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57th meeting of the DPG Working Group
"VIRUSKRANKHEITEN DER PFLANZEN"
March 17th and March 18th 2025

at
KWS, Einbeck

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DPG-working group leaders: Annette Niehl & Björn Krenz

Organization & host: Katia Schütze & Ullrich Dubiella

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Dear colleagues,

We and the team of Katia Schütze & Ullrich Dubiella are welcoming you to the 57th meeting of the DPG Working Group " Viruskrankheiten der Pflanzen" in Einback on Monday, March 17th 2025 and Tuesday March 18th 2025.

KWS SAAT SE & Co. KGaA

Grimsehlstr. 31

37574 Einbeck

We look forward to a meeting that will benefit from numerous and diverse contributions, discussions and questions. The 2025 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.

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How to get to the meeting

By train:
The nearest train exit "Haltestelle Otto-Hahn-Straße" is located behind the KWS premises. From there it is about a 10 minute walk to the main entrance.

By car:
Address for the navigation system:

KWS SAAT SE, Grimsehlstr. 31, 37574 Einbeck

Leave the A7 Hanover-Kassel highway at the Northeim-Nord junction and then turn onto the B3 in the direction of Einbeck.
Leave the B3 at the "Gewerbegebiet Ost" exit. Then take the first right before the Aral petrol station. (KWS is signposted).
At the next set of traffic lights, turn left into Grimsehlstraße towards KWS.

There are only a few parking spaces in front of KWS. Best parking possibility is the parking lot at Barumstr./Grimsehlstr. (orange "P" in picture above)

Entering the premises:
Please enter the campus through our main entrance at Grimsehlstr. 31, and let the doorman know that you are attending the conference.

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Please note that the **cash** 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.

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In order to attend the MS teams meeting online (for free) please follow this link: **Join the 57th AK Virus now**
Meeting ID: 321 905 842 683
Passcode: DQ6Qq9dE

In case of technical problems write an e-Mail to Ullrich.dubiella@kws.com

This link is reserved for conference participants.

Our keynote speakers

Dr. Khalid Amari is a Senior Research Scientist at the Julius Kühn Institute (JKI), Germany, where he works at the Institute for Biosafety in Plant Biotechnology in Quedlinburg. His research focuses on plant-virus interactions, host susceptibility factors, RNA interference (RNAi), virus-induced gene silencing (VIGS), and epigenetic modifications for sustainable disease resistance. With a strong background in plant molecular biology, he has contributed significantly to the field of RNA-based plant defense mechanisms and the development of non-GMO strategies for crop protection. Through his research and collaborations with leading plant biotechnology institutes, Dr. Amari aims to create environmentally friendly solutions to combat plant pathogens while reducing the reliance on chemical treatments. His work is at the forefront of molecular-based plant protection strategies, offering innovative approaches to safeguarding crops against diseases.

In his talk, "*Epigenetics and RNA Interference in Plant Protection: Opportunities, Challenges, and Risks*" Dr. Amari outlined the potential and limitations of RNAi and epigenetics in crop protection. He discussed how exogenous dsRNA application has emerged as a key tool in modern plant protection strategies, effectively suppressing targeted genes to control pathogens. Additionally, he highlighted how epigenetic modifications can turn off susceptibility genes, enabling plants to develop long-term resistance against diseases like powdery mildew and viral infections. However, he also addressed critical challenges such as ensuring specificity, avoiding unintended off-target effects, and navigating regulatory and safety concerns. His presentation emphasized the need for continued research to refine these techniques and integrate them into real-world agricultural applications, offering sustainable solutions to crop protection challenges.

Dr. Samar Sheat is a Postdoctoral Researcher at DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Germany, where she works in the Plant Virus Department. Her research focuses on plant-virus interactions, genetic resistance mechanisms, and molecular breeding approaches for improving cassava's resilience against viral diseases. With expertise in high-throughput screening, genome-wide association studies (GWAS), and transcriptomics, she has significantly contributed to identifying and characterizing resistance genes against Cassava Brown Streak Disease (CBSD) and Cassava Mosaic Disease (CMD). Through her work, Dr. Sheat aims to develop molecular markers and precision phenotyping tools to accelerate cassava breeding programs, providing sustainable solutions to enhance food security in virus-affected regions. By collaborating with global research institutions, she is actively involved in deploying resistant cassava lines to mitigate the impact of devastating plant viruses.

In her talk, *"Towards Disease-Resistant Cassava: Insights from Virus Research Integrating Genetic and Molecular Approaches,"* Dr. Sheat presented her latest findings on cassava's natural resistance against CBSD. She detailed how cassava germplasm from its center of origin in South America revealed distinct resistance patterns, including restricted virus replication and movement. Her research identified key resistance-associated genes, including Ham-1, which plays a crucial role in viral replication and symptom development. Additionally, she discussed the application of GWAS and small RNA sequencing, which implicated NLRR genes on chromosome 11 in CBSV resistance and identified lipid transfer proteins as potential immunity-related factors. Using phylogenetic and co-expression network analyses, she demonstrated how gene clusters regulate resistance responses, paving the way for future breeding strategies. However, she also emphasized the challenges of transferring resistance traits across diverse cassava cultivars and the need for precision phenotyping to improve selection efficiency. Her work provides critical insights into cassava-virus interactions and presents innovative strategies for developing disease-resistant crops, ultimately safeguarding the livelihoods of millions dependent on cassava as a staple food source.

Dr. Werner Beyer is Group Lead of Sugarbeet resistance breeding at KWS, located at KWS headquarters in Einbeck/Germany. After his study of Biology at the University of Tübingen with focus on Plant Virology (Prof. Evamarie Sander), he received his PhD in Plant Breeding and Quantitative Genetics at University of Hohenheim (Prof. H. Geiger) with research on quantitative genetics of *Fusarium* resistance in hybrid rye. He joined KWS in 1995, starting with various research projects and since 1997 as resistance breeder in sugarbeet. Next to resistance breeding programs towards nematodes (*Heterodera schachtii, Meloidogyne* spp.) and fungal root rot diseases (*Rhizoctonia solani, Aphanomyces cochlioides*, and *Fusarium* spp.) he took many years responsibility for the Virus Yellows (BYV, BMYV) resistance breeding program.

Next to resistance breeding programs towards nematodes (*Heterodera schachtii, Meloidogyne* spp.) and fungal root rot diseases (*Rhizoctonia solani, Aphanomyces cochlioides*, and *Fusarium* spp.) he took many years responsibility for the Virus Yellows (BYV, BMYV) resistance breeding program. Since 2008, he is leading the Sugarbeet resistance breeding group, coordinating the resistance variety development worldwide. In his talk "*Virus resistance in sugarbeet – a breeder's perspective*", he will address the status of current breeding programs, the challenges of resistance genetics as well as the combination of Virus resistance with other traits to meet demands for successful market varieties. In addition, he will elaborate on the potential of new breeding methods towards virus resistance.

Programme 2025

Monday, 17. March 2025	
12:30 – 13:15	**Registration with snacks and drinks**
13:15 – 13:25	**Welcome & conference information** *Annette Niehl, Katia Schütze, Ullrich Dubiella & Björn Krenz*
13:25 – 13:30	**Information from the DPG** *Michael Klüken*
	Chair: Katia Schütze & Ullrich Dubiella
13:30– 14:05	**Virus resistance in sugarbeet – a breeder's perspective** *Werner Beyer*
14:05 – 14:20	**Sugar beet resistance to beet mosaic virus (BtMV): phenotypic and transcriptomic findings** *Dennis Rahenbrock*
14:20 – 14:35	**Identification of Immune Response Genes of Cassava During Early Phases of Cassava Brown Streak Virus Infection** *Jessica Lilienthal*
14:35 – 14:50	**Insights into the interaction of the resistance protein Rz2 with triple gene block I from beet necrotic yellow vein virus** *Kristin Benjes*
15:05 – 15:20	**Examining the diversity of the VPg-regions of BaMMV isolates in Germany** *Claudia Strauch*
15:20 – 16:00	**40min coffee & tea break – chair: Mark Varrelmann & Björn Krenz**
16:00 – 16:40	**Epigenetics and RNA Interference in Plant Protection: Opportunities, Challenges, and Risks** *Khalid Amari*
16:40 – 16:55	**Co-infection of beet mosaic potyvirus and beet yellows closterovirus affects virus transmission, localization, accumulation and aphid vector behavior** *Souheyla Khechmar*
16:55 – 17:40	**Poster pitches à 2min** **+ online (4min) by:** • Michael Hagemann: Expanding Host Range of Viroids in Hop: Detection of Hop Stunt Viroid in German Fields and Confirmation of Citrus Exocortis Viroid Pathogenicity • Swati Jagani: Viroid Detection, Simplified: A Sap-Based PCR Solution for Rapid Diagnostics • Clemence Marchal: Novel insights into the potato virus X genome
17:40 – 18:20	**Poster session I & Tour KWS I** • Simon Borgolte: One-step TaqMan® RT-qPCR detection of sugar beet-infecting poleroviruses in *Myzus persicae* collected from yellow water pan traps • Anton Frank: Auftreten und Verbreitung des birch leaf roll-associated virus (BLRaV) und des cherry leaf roll virus (CLRV) in Blattroll-erkrankten Birkenarten an verschiedenen Standorten Europas • Bennet Garz: Investigating the Variability of Barley Yellow Mosaic Virus VPg and Its Interaction with Barley eIF4E Alleles • Jessica Gehrke: Ubiquitin Conjugation Cascade components influence virus-specific disease responses in Arabidopsis • Cornelia Heinze: Progress for the application of virus-induced hypovirulence in the field • Hritu Sarkar: Identification and characterization of new sources of resistance to tomato brown rugose fruit virus (ToBRFV) to generate resistant tomato plants • Swathi Ramapuram: ManiVir : Host and vector manipulation following multi-infection of aphid transmitted viruses in sugar beet (Beta vulgaris) • Ana Osojnik: PVDive: a sneak peek of a new online resource for plant virology
18:20 – 19:00	**Poster session II & Tour KWS II**

	• Ou Sha: Tobamovirus-equipped 3D matrices for biosensing in mini-columns and flow cells • Dennis Knierim: Untersuchung der Viruskrankheiten im Knoblauchanbau auf Mauritius • Dennis Knierim: The value of authenticated and characterized plant virus reference material: the case of potato virus Y in the collection at Leibniz Institute DSMZ • Bruno Mendes: Green synthesis of silver nanoparticles from Dioscorea alata leaves and evaluation of their potential for virus elimination in yam tissue culture • Shaheen Nourinejhad Zarghani: Detection and In Vitro Assessment of Viral Infections in Birch Trees (Betula spp.) • Qiuyu Xu: Exploring the Molecular Mechanisms of Viral Co-Infection Impact on Aphid-Mediated Virus Transmission • Katja Richert-Pöggeler: The tobamovirus sphere of ornamentals illustrated by Hoya, a member of the *Apocynaceae* • Alice Chodura: Comparison of Partial RNA1 Sequences of Ash Shoestring-Associated Virus
19:00	**Conference dinner @ KWS (on site)**

Tuesday, 18. March 2025	
08:15 – 08:30	**Welcome & conference information** *Annette Niehl & Björn Krenz*
	Chair: Laura Medina Puche & Khalid Amari
08:35 – 09:15	**Towards Disease-Resistant Cassava: Insights from Virus Research Integrating Genetic and Molecular Approaches** *Samar Sheat*
09:15 – 09:30	**Viruserkrankungen und die Suche nach Resistenzquellen als Herausforderung für einen erfolgreichen Leguminosenanbau** *Heiko Ziebell*
09:30 – 09:45	**Unguided virus (and other pathogens) detection and discovery from HTS data: successes and pitfalls** *Paolo Margaria*
09:45 – 10:00	**Analyse des Viroms von Kultur- und Wildheidelbeeren in Deutschland mit besonderem Augenmerk auf den sogenannten „Off-Types“** *Wulf Menzel*
30min break Coffee & tea	**Chair: Katja Richert-Pöggeler & Tatjana Kleinow**
10:30– 10:50	**Genetic basis of transgenerational barriers in plant virus vertical transmission** *Gesa Hoffmann*
10:50 – 11:10	**Manipulation of the plant RNA splicing machinery by geminiviruses** *Man Gao*
11:10 – 11:30	**A viral peptide as a trigger for the relocalization of a viral effector** *Laura Medina Puche*
10min break	
11:40 – 12:00	**The role of complementary-sense genes of Beet curly top virus in intermolecular recombination frequency upon local infection in plants** *Omid Eini*
12:00 – 12:15	**Notes from the meeting with GfV** *by Christina Wege & Björn Krenz*
12:15 – 12:30	**Farewell & honors *by*** *Annette Niehl & Björn Krenz* **Coffee & tea and Snacks 2 go**

ABSTRACTS

Monday, 17. March 2025

Keynote

Virus resistance in sugarbeet – a breeder's perspective

Werner Beyer

KWS Saat SE & Co. KGaA, 37574, Einbeck

In his talk "Virus resistance in sugarbeet – a breeder's perspective", he will address the status of current breeding programs, the challenges of resistance genetics as well as the combination of Virus resistance with other traits to meet demands for successful market varieties. In addition, he will elaborate on the potential of new breeding methods towards Virus resistance.

Sugar beet resistance to beet mosaic virus (BtMV): phenotypic and transcriptomic findings

Dennis Rahenbrock[1], Niels Wynant[2], Mark Varrelmann[1], Lukas Rollwage[1]

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Sugar beet (Beta vulgaris) production has historically relied on chemical control measures to manage vectors of viral pathogens, including beet mosaic virus (BtMV; genus Potyvirus). However, the increasing regulatory and environmental constraints on chemical use highlight the need for alternative solutions. In the past, breeding programs for sugar beets have prioritised traits such as sugar yield, and resistance to fungal and bacterial pathogens, with limited efforts placed on virus resistance except rhizomania.

Our study explores quantitative BtMV-resistance in sugar beet which was initially observed in the 1970s in California, USA (Lewellen, 1973). In 2006, Friesen et al. showed, that a single gene, located on chromosome 1 is responsible for the trait. Initial greenhouse biotests revealed that the resistant genotype exhibited virus-like symptoms, albeit with a 10-day delay compared to susceptible plants. However, these symptoms were significantly weaker and appeared as small, lightening-shaped spots on older leaves. RT-qPCR analysis confirmed a substantial reduction in virus titre, depending on the sampling time, with reductions ranging from 7- to 150-fold compared to the susceptible genotype. Furthermore, the inoculation with a GFP-labelled BtMV infectious clone showed minimal or no transport into the phloem, despite comparable virus concentrations within the infiltration patch. To further elucidate the underlying mechanisms, transcriptome analyses were conducted to identify genes potentially responsible for the observed phenotype. By comparing BtMV-infected resistant and susceptible genotype with mock-inoculation, 1130 differentially expressed genes (DEGs) could be identified in the mock and BtMV-treated resistant genotype, 419 of which were upregulated, while 711 were downregulated by at least threefold. The most pronounced changes were observed in regulatory processes related to transport, external stimuli, RNA biosynthesis and protein modification.

By uncovering the genetic basis of the resistance, this research not only advances our understanding of BtMV resistance but also underscores the potential for integrating such traits into future breeding programs.

Literature

- Friesen, T. L.; Weiland, J. J.; Aasheim, M. L.; Hunger, S.; Borchardt, D. C.; Lewellen, R. T. (2006): Identification of a SCAR marker associated with Bm, the beet mosaic virus resistance gene, on chromosome 1 of sugar beet. In: Plant Breeding 125 (2), 167–172.
- Lewellen, R. T. (1973): Inheritance of Beet Mosaic Virus Resistance in Sugarbeet. In: Phytopathology 63, 877–881

Identification of Immune Response Genes of Cassava During Early Phases of Cassava Brown Streak Virus Infection

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The cassava brown streak disease (CBSD), caused by the ipomoviruses CBSV and UCBSV, poses a significant threat to cassava crops, with the destruction of tuberous roots resulting in substantial yield losses. The virus-resistance identified in some South American cassava germplasm lines inhibits virus replication and restricts virus movement to the phloem companion cells, resulting in complete immunity in some lines or a restriction of the infection to the root only. To further explore resistant genotypes and identify potential resistance genes, a time course RNA-seq study was conducted, comparing gene expression of a virus-susceptible cassava TMS 96/0304, a CBSV root-restricting cassava DSC 260 and an immune DSC 167 cassava. Differential gene expression patterns across leaves, stems, and roots revealed substantial differences between the susceptible and resistant genotypes. Temporal analysis revealed significant variations in the number of differentially expressed genes (DEGs), particularly in the stem at one day after inoculation (DAI), with notable expression observed in DSC 167 (436 DEGs) and DSC 260 (335 DEGs). Significant expression changes were noted in the virus-infected TMS 96/0304 (737 DEGs) only at 10 DAI. Several DEGs in resistant genotypes were associated with proteins involved in phenylpropanoid biosynthesis, protein processing in endoplasmic reticulum, plant hormone signal transduction, and plant-pathogen interactions. The analysis indicates that virus resistance in the South American resistant lines is driven by early-stage regulation of genes in the classes of heat shock proteins and auxin proteins. Validating these genes will present a step forward to understand virus resistance and accelerate CBSV resistant screening in germplasm and segregating populations.

Insights into the interaction of the resistance protein Rz2 with triple gene block I from beet necrotic yellow vein virus

Benjes K, Varrelmann M, Liebe S

Institute of Sugar Beet Research Göttingen

The disease rhizomania is caused by beet necrotic yellow vein virus (BNYVV). Control relies on the resistance genes Rz1 and Rz2. Occurrence of Rz1 resistance-breaking populations emphasizes the importance of Rz2. The encoded protein has been identified as nucleotide binding (NB) and leucine-rich-repeat receptor (Capistrano-Gossmann et al., 2017). In a transient assay in Nicotiana benthamiana, Rz2 was shown to recognise BNYVV triple gene block I (TGB1) as corresponding avirulence protein leading to a hypersensitive response (HR) with cell death (Wetzel et al., 2021).
Using a K201A Rz2 mutant to abolish the rapid HR, it was shown that Rz2 and BNYVV TGB1 colocalise in the cytoplasm and nucleus. Manipulation of the subcellular localisation by fusion of wild-type Rz2 and BNYVV TGB1 with a nuclear localisation signal resulted in a greatly reduced HR whereas fusion of both with a nuclear export signal did not affect the HR. This suggests that the cytoplasmic distribution of both is important for Rz2 mediated resistance. To test for a direct interaction, a yeast-two hybrid (Y2H) assay and bimolecular fluorescence complementation in planta using a K201A Rz2 mutant and a L8A, L14A, L18A Rz2 mutant were conducted. Both failed to show a direct physical interaction which suggests an indirect interaction with a conserved host protein present in Beta vulgaris as well as in N. benthamiana. Optimisation for proximity labelling is ongoing to identify a candidate intermediate host protein as well as other unknown interaction partners.
To enclose the region in BNYVV TGB1 that is crucial for recognition by Rz2, deletion constructs were tested. Two conserved motifs were identified that are sufficient to initiate a HR and in which mutations could lead to circumvention of recognition.

Literature

- Capistrano-Gossmann, G. G., Ries, D., Holtgräwe, D., … Kopisch-Obuch, F. J. (2017). Crop wild relative populations of Beta vulgaris allow direct mapping of agronomically important genes. Nature Communications, 8(1), 15708. https://doi.org/10.1038/ncomms15708
- 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(7), 829–842. https://doi.org/10.1111/mpp.13066

Examining the diversity of the VPg-regions of BaMMV 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, while infection with BaMMV is described to have less impact on plant health. To avoid crop losses due to the viruses, resistant barley cultivars are used by farmers. The two barley resistance genes rym4 and rym5 are intensively used in resistance breeding. Interestingly, rym4 and rym5 are coding for different alleles of the eukaryotic translation initiation factor 4E (eIF4E). Against BaMMV, the rym4 resistance is very durable in the field, with no resistance breaking isolates observed until now. However, BaMMV isolates can overcome the rym5 resistance. We monitor fields in northern Germany to analyze the frequency of resistance breaking. Additionally, we focus on the viral VPg-region as an important factor, which can determine resistance-breaking. We expect to obtain insight into the viral diversity and adaptation of virus isolates to the presence of resistant plants. With this information, we hope to get a better understanding of the durability of resistance to support barley breeders in the future.

Epigenetics and RNA Interference in Plant Protection: Opportunities, Challenges, and Risks

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RNA interference (RNAi) and epigenetics are key biological processes that regulate gene expression and protein synthesis in plants, particularly during development and stress responses. The exogenous application of double-stranded RNA (dsRNA) has emerged as a powerful tool for inducing systemic gene silencing, effectively suppressing specific genes across various tissues and organs. This technique has shown great promise as a plant protection strategy against pathogens such as viruses, fungi, and insect pests.

Epigenetics, on the other hand, allows plants to regulate gene expression dynamically, enabling rapid adaptation to environmental stresses. Pathogens rely on host susceptibility factors for successful infection, and targeting these factors through epigenetic modifications can confer resistance. In our research, we have demonstrated the successful use of epigenetics to silence susceptibility genes for viruses and powdery mildew in Arabidopsis and tobacco plants. Using techniques such as virus-induced gene silencing (VIGS) and the SunTag system coupled with methyltransferase, we stably methylated promoter regions of selected susceptibility genes, leading to their downregulation. Our results show that these epigenetic modifications can provide resistance to pathogens, and remarkably, these effects can be inherited by subsequent plant generations, even in the absence of transgenes.

Despite the potential of RNAi and epigenetics in plant protection, challenges remain, including ensuring specificity, minimizing unintended effects, and addressing regulatory and safety concerns.

Co-infection of beet mosaic potyvirus and beet yellows closterovirus affects virus transmission, localization, accumulation and aphid vector behavior

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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 co-infected by several viruses, among them the non-circulative, semi-persistent, phloem-limited beet yellows virus (BYV, Closteroviridae), and the non-circulative, non-persistent, non-phloem-limited beet mosaic virus (BtMV, Potyviridae) that were studied here and which share the same vector, the green peach aphid (Myzus persicae). BYV/BtMV co-infected plants exhibited more pronounced growth stunting and mosaic symptoms compared to single virus infection. Aphid transmission of BYV from co-infected sugar beet was reduced by 50%, while BtMV transmission was not impacted. RT-qPCR analysis showed a significant increase in accumulation of both viruses in co-infected plants, suggesting that viral titre does not explain the reduced BYV transmission. Electropenetrography experiments showed that the drastic decrease of BYV transmission was also not due to reduced phloem sap ingestion by aphids from co-infected plants. Virus localisation experiments by fluorescent in situ hybridization (SABER-FISH) showed that co-infection did not relieve phloem restriction of BYV. Also BtMV tissue distribution was unaffected by co-infection. BtMV accumulated in periplasmic aggregates in mono-infected and co-infected cells, while BYV formed cytoplasmic spherical inclusions in mono-infected cells and displayed a granular and more diffuse distribution in co-infected cells. This indicates BYV-BtMV interactions in co-infected plants. We propose that these interactions could restrict BYV access to the sieve tubes from where BYV is acquired and thus reduce its accessibility for aphids and present a model how BtMV could impede BYV phloem loading and reduce transmission.

Towards Disease-Resistant Cassava: Insights from Virus Research Integrating Genetic and Molecular Approaches

Sheat S, Winter S

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In the dynamic field of cassava (*Manihot esculenta*) research, our focus is on understanding cassava diseases worldwide to provide control options and mitigate the impact of cassava diseases. We base our work on an extensive and steadily growing collection of viruses and virus-like agents from South America, Africa, Brazil, and Asia and the worldwide collaboration of cassava research institutes.

In the past years, our virus work was focused on combating the severe Cassava Brown Streak Disease (CBSD) which is a critical threat to food security in Sub-Saharan Africa. Utilizing cassava's center of origin in South America, we identified and characterized resistant cassava lines, defined resistance patterns based on restriction of virus replication and restriction of virus movement, and we deployed these valuable genetic resources to epicenters of the disease in Eastern and Central Africa and to regions at risk for pre-emptive breeding. On this basis, we now integrate additional traits to enhance cassava's resilience against biotic stresses and adverse environmental conditions.

To understand virus responses of cassava, cDNA clones of cassava brown streak viruses and modified virus genomes were used to infect cassava and *Nicotiana benthamiana* to unravel viral and host genes associated with resistance responses. Notably, the *Ham-1* gene was identified as a pivotal player for cassava infections implicated in virus replication, movement, symptom expression, and resistance in cassava.

To further understand plant resistance, we conducted Genome-Wide Association Studies (GWAS), mapping by sequencing, and small RNA analysis on resistant and susceptible plants. Weak signals from GWAS and GBS on chromosome 11 implicated *NLRR* genes as potential contributors to CBSV resistance. Additional signals on chromosome 3 were associated with UCBSV resistance, warranting further verification. Small RNA sequencing further identified novel miRNAs implicating lipid transfer genes as potential players in plant immunity. Preliminary proteomic data provided evidence for a lipid-binding protein as candidate for resistance, while transcriptomic data highlighted heat shock proteins as important candidates in early stages of cassava infection.

To investigate the genetic relationships between South American cassava lines and those from other regions, we constructed a phylogenetic tree, which revealed distinct clustering patterns. Additionally, weighted gene co-expression network analysis (WGCNA) using public RNA-seq data indicated co-expression of gene modules that warrant further investigation. These findings contribute to our understanding of viral and host factors regulating resistance to viruses infecting cassava.

Through our innovations, we aim to protect cassava—a staple food for millions of people—and contribute to safeguarding the livelihoods of communities that depend on this essential crop.

Viruserkrankungen und die Suche nach Resistenzquellen als Herausforderung für einen erfolgreichen Leguminosenanbau

Ziebell H

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Leguminosen sind wichtige Nutzpflanzen, da sie Nahrungsmittel für Mensch und Tier liefern, als Zwischenfrüchte zur Verbesserung der Bodengesundheit oder als Gründüngung verwendet werden können, da sie aufgrund der symbiotischen Beziehung zu Rhizobakterien atmosphärischen Stickstoff fixieren können. In der Vergangenheit lag der Schwerpunkt bei der Züchtung von Leguminosen auf der Anpassung an klimatische Bedingungen (z. B. Kälteresistenz von Ackerbohnen), der Reduzierung unerwünschter Stoffwechselprodukte (Vicin/Convicin bei Ackerbohnen, Alkaloide bei Lupinen) oder der Resistenz gegen Schädlinge und Krankheitserreger, vor allem gegen pilzliche Krankheitserreger (Anthraknose bei Lupinen, Aphanomyces-Wurzelfäule bei Erbsen, Fusarium bei Erbsen, usw.).
Im Gegensatz dazu war die Resistenzzüchtung gegen virale Krankheitserreger bei vielen Leguminosen bisher ein untergeordnetes Zuchtziel. Die meisten Arbeiten zur Einführung von Virusresistenzen in Leguminosensorten wurden bei Erbsen durchgeführt, z. B. die Einführung von Resistenzen gegen das Erbsenenationsmosaikvirus (PEMV) oder das Blattrollvirus (BLRV). Die große Anzahl an leguminosen-infizierenden Viren erschwert die die Selektion von Resistenzquellen und damit die gezielte Virusresistenzzüchtung. Darüber hinaus treten auch neue Pflanzenviren an Leguminosen auf, wie seit 2011 in Deutschland das pea necrotic yellow dwarf virus (PNYDV). Die von Viren verursachten Schäden sind vielfältig und können direkte Ertrags- und Qualitätsverluste hervorrufen (Saucke et al. 2019. 10.1007/s10658-018-01643-5), die symbiontischen Beziehungen von Leguminosen zu Rhizobakterien stören (Seeger et al. 2022. 10.1007/s41348-022-00646-z) und das Verhalten von Blattlausvektoren beeinflussen (Berawe et al., 2024) und Kosten für die Bereitstellung von virusfreiem Saatgut verursachen. Da es keine Behandlungsmöglichkeiten von Viruserkrankungen gibt, bieten virusresistente Pflanzen den besten Schutz. Die Suche nach geeigneten Resistenzquellen ist insbesondere für blattlausübertragene Viren aufwändig, bietet jedoch das Potential, virusresistente Sorten zu züchten, die für einen nachhaltigen und erfolgreichen Leguminosenanbau zwingend erforderlich sind.

Unguided virus (and other pathogens) detection and discovery from HTS data: successes and pitfalls

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The potential and benefits of high-throughput sequencing for pathogen diagnostics are well-known and accepted. A major bottleneck relays however on data analysis: sample sequencing is often outsourced, while generated data are thereafter processed *in house*, requiring appropriate resources and specific expertise to conduct the bioinformatic analyses and assess the results for proper identification. Nevertheless, (semi-)automated pipelines may be of significant support to conduct unguided analyses and provide a first overview, paving the path to more detailed investigations. The experience gained with the PhytoPipe pipeline, an open-source bioinformatic pipeline recently developed for comprehensive plant pathogens detection and implemented in the USDA Plant Germplasm Quarantine Program for HTS-based diagnostics, will be illustrated considering plant materials with various pathogen profiles, benchmarked to the standard discovery pipeline in use in our laboratories. Further, implementation of PhytoPipe on a high-end notebook will be discussed, together with novel approaches based on machine learning to overcome the need of computationally-intense reference database generation.

Acknowledgements

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Analyse des Viroms von Kultur- und Wildheidelbeeren in Deutschland mit besonderem Augenmerk auf den sogenannten „Off-Types“

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Die Kulturheidelbeeren (*Vaccinium corymbosum* Sorten) ist in Deutschland eine hochwertige Kulturpflanze und Trendfrucht, deren wirtschaftliche Bedeutung in den letzten Jahren stark zugenommen hat. Die Anbaufläche hat sich in den letzten 15 Jahren mehr als verdoppelt und betrug 2023 rund 3.500 ha. Ab der Saison 2015 wurden in mehreren Plantagen in Norddeutschland Unregelmäßigkeiten im Ertrag einzelner Heidelbeerpflanzen festgestellt. Auffällig war, dass diese Pflanzen alle aus der Anbausaison 2012/13 stammten. Das sortenuntypische Verhalten wurde vor allem bei der wichtigen Sorte 'Duke', aber auch bei anderen Sorten wie 'Draper' und 'Liberty' beobachtet.
So zeigte die wichtige frühe Sorte 'Duke' einen weit ausladenden Wuchs, verkürzte Internodien, kleinere Beeren und eine unvollständige Ablösung der Blütenblätter. Off-Type Pflanzen stehen oft neben ertragreichen Pflanzen aus der gleichen Lieferung und Sorte. Zahlreiche Versuche, die Ursache zu klären, blieben bisher erfolglos. Bislang muss in Europa gehandeltes Jungpflanzenmaterial nur Mindestanforderungen an die Sortenechtheit und die äußere Krankheitsfreiheit erfüllen; solche atypischen Verhaltensweisen, die häufig erst nach mehreren Jahren in Bestand augenscheinlich werden, oder latente Virusinfektionen werden dabei nicht berücksichtigt. Mehr als ein Dutzend Viren sind für die Heidelbeere bekannt, und die meisten wurden ursprünglich in Nordamerika gefunden. Über das Vorkommen von Heidelbeerviren in Europa ist wenig bekannt, aus Deutschland fast nichts. In einem Verbundprojekt werden nun verschiedene mögliche Ursachen für Off-Types untersucht, mit dem Ziel, Marker für eine frühzeitige Erkennung im Vermehrungsmaterial als Zertifizierungskriterium zu identifizieren. Dazu gehören neben der Untersuchung der Gewebekulturbedingungen und dem Einsatz von Hormonen auch mögliche epigenetische Veränderungen im Zusammenhang mit der In-vitro-Vermehrung. Darüber hinaus wird das Virom der Heidelbeere in Deutschland untersucht um mögliche Korrelationen zu identifizieren. Dieser Vortrag fasst die allgemeine Situation und die bisherigen Ergebnisse der virologischen Arbeitspakete zusammen. Die Untersuchungen mittels Illumina Hochdurchsatzsequenzierung haben bisher zur Identifizierung von vier Viren geführt. Das blueberry mosaic virus (BlMaV) und das blueberry latent virus (BBLV) wurden erstmal ins Deutschland nachgewiesen und das blueberry mitovirus und das blueberry virus L (BlVL) stellen den ersten Nachweis für Europa dar. Die bisherigen Virusnachweise zeigen jedoch keine Korrelation mit den beobachteten Off-Type Symptomen. In den untersuchten Wildheidelbeer-Mischproben (Vaccinium myrtillus) konnten bisher keine Viren identifiziert werden.

Genetic basis of transgenerational barriers in plant virus vertical transmission

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To ensure their continued existence viruses must not only master their replication within one host, but also the transmission between hosts. Plant virus transmission can be divided into two broad categories: horizontal and vertical. During horizontal transmission the virus moves between susceptible hosts, often mediated by insect or nematode vectors. Vertical transmission, on the other hand, occurs when an infected parent plant generates infected progeny, meaning the virus has successfully maintained infection through the plants sexual reproduction and passed the transgenerational barrier. Vertical transmission can lead to rapid worldwide spread of disease through seed trade and/or local spread through pollen. Understanding how vertical virus transmission is controlled in plants is therefore critical to prevent infection and its consequences.

By establishing Turnip yellow mosaic virus as our model, we are fine-mapping the timing, localization and mechanisms of transgenerational barriers within the *Arabidopsis thaliana* germline. We have identified AGO5 as a germline specific antiviral RNAi factor that successfully restricts virus transmission within both, the male and female germline. Through a series of zygosity-dependent crosses, we could narrow down the effective antiviral capacities of AGO5 to specific developmental stages during germline maturation. Cell-type specific complementations of either AGO5 or virus-specific smallRNA-production in the mutant background can partially restore the vertical transmission restriction. This work provides the basis for large-scale genomic screens to identify additional components in establishing the potent host transgenerational barriers and could shine a light on unknown cell type-specific antiviral defense mechanisms

Manipulation of the plant RNA splicing machinery by geminiviruses

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Geminiviruses are a large family of plant DNA viruses that poses a serious threat to agriculture and food security worldwide. Geminiviruses replicate in the nuclei of the infected cells, and due to their limited coding capacity, they rely heavily on the host machinery for replication and spread. While studies in mammalian viruses have uncovered splicing as a key host mechanism hijacked during infection, it remains elusive if geminiviruses generally take advantage of this process to increase their transcriptome and proteome diversity. We have recently found that i) the geminiviral replication-related protein, Rep, physically associates with the plant splicing machinery; ii) geminiviral transcripts are spliced and give rise to new proteins with functional relevance during infection; iii) splicing is required for geminiviral replication and systemic infection; and iv) the geminiviral infection drastically impacts the host splicing landscape. Therefore, splicing emerges as a central process at the interface between geminiviruses and their host plants. We have identified one Rep-associated splicing factor, MAC3A (MOS4-Associated Complex 3A), which encodes a U-box protein homologous to the yeast and human E3 ubiquitin ligase Prp19, as essential for viral infection. We propose that viral Rep may manipulate the function of MAC3A in RNA splicing to promote virus infection.

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 C4G2A 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. Our findings suggest that PVG functions as a viral PAMP mimic, possibly being perceived by the LRR-RP NbRE02 to trigger the re-localization of the viral virulence factor C4 to a second site of action.

The role of complementary-sense genes of Beet curly top virus in intermolecular recombination frequency upon local infection in plants

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Geminiviruses are suitable models for studying DNA replication and recombination. Intermolecular DNA recombination is a critical process in the evolution of geminiviruses. In this study we investigated the effect of viral factors on homologous recombination frequency (HRF) in a model system between replicons of Beet curly top virus which contains key genes for the virus replication (called BCTVRepl) and Cotton leaf curl Multan betasatellite which lacks the βC1 gene (called CLCB) in locally co-infected plant tissues. A fluorescence-based screening method was developed to test the recombination between the C-terminus and N-terminus of the GFP marker gene which were subcloned into BCTVRepl and CLCB replicons to produce BCTVRepl-cGFP and CLCB-nGFP, respectively. The introduction of premature stop codons in the C2 and C4 genes of BCTVRepl-cGFP resulted in a substantial reduction in HRF without affecting the accumulation of BCTVRepl-cGFP. This finding suggests that C4 and to a greater extent C2 play a role in HR between BCTV and CLCB replicons. In addition, mutations in the BCTV large intergenic region (LIR) sequence to which C1 (Rep) protein binds and initiates viral replication showed that the iteron and stem-loop sequences within the LIR are essential for intermolecular recombination. This study presents a new model for HRF assessment in BCTV that can be applied to other geminiviruses and highlights the role of C2, C4, C1 and LIR structural sequences in intermolecular recombination and replication in BCTV

Poster abstracts

Expanding Host Range of Viroids in Hop: Detection of Hop Stunt Viroid in German Fields and Confirmation of Citrus Exocortis Viroid Pathogenicity

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Humulus lupulus L., commonly known as hop, is primarily cultivated for brewing but has gained increasing interest for its applications in pharmaceuticals and bioeconomic innovations. Currently, five viroids are known to infect hops: the severe stunting pathogen *citrus bark cracking viroid* (CBCVd), the stunting viroids *hop stunt viroid* (HSVd) and *apple fruit crinkle viroid* (AFCVd), the latter until now only reported in Japan, the mostly symptomless *hop latent viroid* (HLVd), and *citrus exocortis viroid* (CEVd), identified as infectious in greenhouse experiments.

In this study, we report the detection of HSVd in commercial hop fields in Southern Germany, likely originating from HSVd-infected wild grape plants introduced as part of hop field refugia for beneficial insects and predatory mites. To our knowledge, the extent of this infection appears to be contained to a single location.

Furthermore, in 2023, we demonstrated that CEVd can be transferred from citrus fruit peels to hop plants. By examining the growth of CEVd-infected and non-infected control plants, we confirmed that this viroid can cause stunting, at least under experimental conditions. Tall, potted plants of the cultivar 'Herkules' showed a significant reduction in biomass during the growth season following a dormancy period, verifying the threat posed by this globally distributed citrus viroid.

We compare current knowledge on the pathogenicity of these hop-infecting viroids and explore potential similarities in their mechanisms of action. These findings emphasize the need for proactive viroid management strategies to safeguard the expanding roles of hops in agriculture and industry.

Viroid Detection, Simplified: A Sap-Based PCR Solution for Rapid Diagnostics

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Viroids are infectious, small, non-coding RNAs that pose a significant threat to agriculture. Traditional detection methods rely on RNA extraction from plant tissue, which is time-consuming and impractical for field diagnostics. This limits early detection, which is critical for effective disease management. This highlights the need for a direct viroid detection method that bypasses RNA extraction, which could greatly improve real-time monitoring and disease control in affected crops.

In this study, we developed a streamlined approach for detecting the destructive Cocadviroid rimocitri (formerly known as citrus bark cracking viroid, CBCVd) directly from leaf sap, bypassing the RNA extraction step entirely. PCR assays using freshly prepared leaf sap successfully amplified CBCVd RNA, demonstrating that this method is a promising alternative for viroid detection.

This method reduces cost and processing time, making it more accessible for rapid diagnostics in resource-limited settings. Eliminating the RNA extraction step enhances its practicality for field applications, where rapid results are critical. The method is also compatible with portable laboratory setups, such as the Bento Lab, allowing for on-site testing without the complex sample preparation.

In conclusion, the sap-based PCR approach developed in this study provides a practical and accessible alternative to RNA-based diagnostics. Its adoption could improve field disease management by enabling real-time pathogen detection and early intervention.

Novel insights into the potato virus X genome

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Although viral genomes are constrained by size, viruses excel at maximising their coding space and manipulating host cells to enable replication and spread. Recently, our lab identified new open reading frames (ORFs) in the tomato yellow leaf curl virus (TYLCV) genome that had been overlooked due to their small size. Several of these ORFs are translated into proteins during infection and are involved in the viral cycle. However, whether small ORFs have been missed in other viral genomes remains unclear. Here, we examined the genome of potato virus X (PVX), a positive-sense single-stranded RNA virus that infects Solanaceae species such as potato, tomato, pepper or the model plant *N. benthamiana*. Remarkably, we identified 19 conserved ORFs across several PVX strains, most of which reside on the negative strand, traditionally considered non-coding. Functional analysis of six ORF knock-out mutants revealed diverse phenotypes upon infection in *N. benthamiana*, ranging from milder symptoms to constitutive cell-death. Notably, three mutants additionally showed reduced viral loads. We are now investigating the function of these newly identified ORFs. These findings suggest that we may need to revisit viral genomes to uncover overlooked genetic elements.

One-step TaqMan® RT-qPCR detection of sugar beet-infecting poleroviruses in *Myzus persicae* collected from yellow water pan traps

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Since the ban of neonicotionoid seed treatment in 2018, virus yellows disease (VY), caused by beet yellows virus (BYV), beet chlorosis virus (BChV) & beet mild yellowing virus (BMYV), has been a major threat to sugar beet production in Europe. BChV and BMYV are of particular economic importance and are both persistently transmitted by the aphid vector *Myzus persicae* (Borgolte et al., 2024; Hossain et al., 2021; Stevens et al., 2004). As part of integrated pest management, *M. persicae* influx into sugar beet fields is recorded weekly using yellow water pan traps. To date, only ELISA and RT-PCR assays have been described for BChV and BMYV detection in individual aphids (Smith et al., 1991; Stevens et al., 1995, Viganó and Stevens, 2007). In addition, no method has been described for the detection in pooled samples of *M. persicae* from yellow water pan traps (7d incubation in water with detergent).

We designed two one-step TaqMan® RT-qPCR assays for the specific detection of BChV and BMYV in *M. persicae* from plants and after 7d incubation in water pan trap medium (water with detergent). Under controlled conditions both viruses were reproducibly detected in individual winged and wingless aphids. After 7d incubation in trap medium, both viruses were reproducibly detected in individual aphids (winged and wingless), but a loss of sensitivity was observed. Furthermore, one viruliferous aphid was detecable in a pool of 99 non-viruliferous aphids after 7d incubation in trap medium. Significant correlations can be shown between different mixing ratios of viruliferous to non-viruliferous aphids and Ct values of total RNA templates, allowing the percentage of viruliferous aphids in yellow water pan traps to be estimated using a standard curve (Borgolte et al. 2025). The described method provides a high sensitivity combined with a high sample throughput and has been tested in large-scale monitoring activities in Germany in 2023 and 2024.

The work was supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) through the project "EntoProg" (funding code 2821ABS032) and partly through the project "Estimation of the risk of infestation by sugar beet yellowing viruses – Foresighted development of control strategies taking into account the neonicotinoid and insecticide resistance problem of the insect vector" (funding code 2814901615).

LITERATURE

Borgolte, S., Menzel, W., Varrelmann, M. (2025) One-step TaqMan® RT-qPCR detection of sugar beet-infecting poleroviruses in Myzus persicae from yellow water pan traps opens up new possibilities for early risk assessment of virus yellows disease. *Journal of Virological methods* 331, 115052.

Borgolte, S., Varrelmann, M., Hossain, R. (2024) Time point of virus yellows infection is crucial for yield losses in sugar beet, and co-infection with beet mosaic virus is negligible under field conditions. *Plant Pathology* 73, 2056-2070.

Hossain, R., Menzel, W., Lachmann, C., Varrelmann, M., (2021) New insights into virus yellows distribution in Europe and effects of beet yellows virus, beet mild yellowing virus, and beet chlorosis virus on sugar beet yield following field inoculation. *Plant Pathology* 70, 584-593.

Smith, H.-G., Stevens, M., Hallsworth, P.-B. (1991) The use of monoclonal antibodies to detect beet mild yellowing virus and beet wester yellows virus in aphids. *Annals of Applied Biolgy* 119, 295-302.

Stevens, M., Hallsworth, P.-B., Smith H.-G. (2004) The effects of Beet mild yellowing virus and Beet chlorosis virus on the yield of UK field-grown sugar beet in 1997, 1999 and 2000. *Annals of Applied Biolgy* 144, 113-119.

Stevens, M., Smith, H.-G., Hallsworth, P.-B. (1995) Detection of the luteovirus, beet mild yellowing virus and beet western yellows virus in aphids caught in sugar beet and oilseed rape crops, 1990-1993. *Annals of Applied Biolgy* 127, 309-320.

Viganó, F., Stevens, M. (2007) Development of a multiplex immunocapture RT-PCR for simultaneous detection of BMYV and BChV in plants and single aphids. *Journal of Virological Methods* 146, 196-201

Auftreten und Verbreitung des birch leaf roll associated virus (BLRaV) und des cherry leaf roll virus (CLRV) in Blattroll-erkrankten Birkenarten an verschiedenen Standorten Europas

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Die Birkenblattrollerkrankung (birch leaf roll disease, BLRD) tritt zunehmend seit der ersten Beschreibung in Finnland 2002 in weiteren Ländern Europas, darunter Deutschland, Frankreich, Schweden und Norwegen in Betula pubescens und Betula pendula auf. Einige Viren konnten bereits mit der Erkrankung assoziiert werden, wobei das Birch leaf rollassociated virus (*Badnavirus volubetulae*, BLRaV) sowie das cherry leaf roll virus (*Nepovirus avii*, CLRV) bisher am häufigsten in den erkranken Birken detektiert wurden (von Bargen et al. 2009; Rumbou et al. 2020; Rumbou et al. 2021; Büttner et al. 2023). Als Verursacher der Symptome der BLRD in Form von Blattrollen, chlorotischen Adernbänderungen und Nekrosen der Blätter bis hin zum Absterben der Bäume wird das BLRaV angenommen, wobei die Symptome durch eine Mischinfektion z.B. mit dem CLRV ggf. verstärkt werden können (Büttner et al. 2023; Rumbou et al. 2021; Vainio et al. 2024). In dieser Studie wurden Blattproben von BLRD-erkrankten Birken an dreizehn Standorten aus Deutschland, Estland, Finnland, Italien, Lettland, Litauen und Polen mittels Virus-spezifischer RT-PCR auf eine BLRaV bzw. CLRV Infektion untersucht. Es wurden Proben von *Betula pendula* (Hängebirke), *B. pubescens* ssp. *pubescens* (Moorbirke), *B. pubescens* ssp. *czerepanovii* (Bergbirke), *B. pubescens* ssp. *appressa* (Kiilopää Birke) sowie *Betula nana* (Zwergbirke) in die Untersuchungen einbezogen. Ergebnisse zum Auftreten in den genannten Birkenarten und zur geographischen Verbreitung beider Viren werden vorgestellt und bewertet.

LITERATUR

von Bargen, S., Grubits, E., Jalkanen, R., Büttner, C. (2009): Cherry leaf roll virus – an emerging virus in Finland? Silva Fennica 43, 727-738. [http://urn.fi/URN:NBN:fife2016101225088]

Büttner C, Landgraf M, Fernandez H, von Bargen S, Bandte M (2023) Virus diseases of forest and urban trees. In: Asiegbu F, Kovalchuk A (eds) Forest Microbiology, Volume 3. Elsevier, Amsterdam, pp 61-97. https://doi.org/10.1016/B978-0-443-18694-3.00011-0

Rumbou A, Candresse T, Marais A., Svanella-Dumas L, Landgraf M, von Bargen S, Büttner C, (2020): Unravelling the virome in birch: RNA-Seq reveals a complex of known and novel viruses. PLos ONE 15(6): e0221834, doi: https://doi.org/10.1371/journal.pone.0221834.

Rumbou, A., Vainio, E.J., Büttner, C. (2021): Towards the Forest Virome: High-Throughput Sequencing Drastically Expands Our Understanding on Virosphere in Temperate Forest Ecosystems. Microorganisms, 9, 1730. https://doi.org/10.3390/microorganisms9081730

Vainio EJ, Rumbou A, Diez JJ, Büttner C (2024): Forest Tree Virome as a Source of Tree Diseases and Biological Control Agents. Current Forestry Reports, doi: [10.1007/s40725-024-00214-8]

Investigating the Variability of Barley Yellow Mosaic Virus VPg and its Interaction with Barley eIF4E Alleles

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The barley yellow mosaic disease, caused by the Barley yellow mosaic virus (BaYMV), leads to substantial yield losses in infected fields. For successful infection, the viral genome-linked protein (VPg) of BaYMV interacts with the host eukaryotic translation initiation factor 4E (eIF4E), thereby recruiting the host translation machinery. Barley's rym4 and rym5 resistance genes encode distinct eIF4E variants, which do not interact with viral VPg. However, certain BaYMV isolates have evolved mechanisms to overcome this resistance, suggesting viral adaptation.
In this study, we analyze the diversity of VPg sequences in BaYMV isolates collected in 2024 to identify sequence variations associated with resistance adaptation. By investigating the natural variation among VPg sequences and their interactions with different eIF4E alleles, we aim to gain insight into viral evolution and host adaptation. Our findings could provide valuable insights for improving resistance-breeding strategies and ensuring long-lasting resistance of barley cultivars against BaYMV.

Ubiquitin Conjugation Cascade components influence virus-specific disease responses in Arabidopsis

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Selective protein catabolism is essential during development or stimuli responses, enabling proteostatic shifts alongside transcriptional and translational regulation. In eukaryotes, the Ubiquitin-Proteasome System (UPS) is the main pathway for selective protein degradation and serves as a central regulatory hub in various cellular processes, including plant immunity and antiviral responses. Consequently, plant viruses have evolved mechanisms to evade or manipulate the UPS to their advantage. The UPS has two phases: (1) the ubiquitination of target proteins via a cascade involving E1, E2, and E3 enzymes, and (2) the degradation of ubiquitin-tagged proteins by the 26S proteasome. The Arabidopsis thaliana genome encodes two E1, ~40 E2, and over 1,500 E3 enzymes, ensuring a rapid, versatile, and substrate-specific response. Our overarching aim is to uncover UPS regulations in viral infections. Here, we observed increased but variable levels of ubiquitinated proteins with different viruses (CaMV, TuMV and CMV), suggesting distinct regulation of the UPS during each infection. To identify the responsive UPS components and targets involved, we performed GFP-ubiquitin immunoprecipitations from infected and healthy Arabidopsis plants, followed by mass spectrometry analysis. We focused on three UPS components enriched in infections: UBA1 (E1 enzyme) and UBC35/UBC36 (E2 enzymes). The *uba1* mutant showed reduced disease severity for all viruses, while the *ubc35/36* double mutant exhibited biomass reduction and necrosis. Furthermore, CaMV accumulation decreased in both mutants, TuMV in *ubc35/36* and CMV in *uba1*. These findings suggest that all three UPS factors influence disease outcomes with a degree of virus-specificity.

Progress for the application of virus-induced hypovirulence in the field

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The ascomycete *Fusarium graminearum* is the main cause of fusarium head blight disease (FHB) in wheat, barley, and corn (ear rot). The infection of crops leads to massive yield and quality loss worldwide every year. The mycotoxins produced by the pathogen also cause severe health problems in humans and animals. Besides, control of the pathogen with fungicides is inefficient; therefore the development of alternatives is of high importance.
The infection of the fungus with *Fusarium graminearum* virus-china 9 (FgV-ch9, *Chrysoviridae*) leads to a reduced systemic spread and an overall reduced pathogenicity, called hypovirulence. Therefore, virus-infected Fusarium strains could be used as an alternative to fungicides. Ideally, virus-infected conidia would be applied in the field. However, each of these conidia would have to contain the virus and germinate into mycelium with a hypovirulent phenotype. In this study, we confirmed unpublished data that the conidia produced by virus-infected mycelium germinate only to 35% into hypovirulent mycelium.
The transmission rate of virus into conidia dramatically increased upon SO overexpression or by knocking out the fungal gene silencing system. The latter will enable us, by the use of genome editing, to modify the fungus with minimal nucleotide changes for the application of this system in the field.

Identification and characterization of new sources of resistance to tomato brown rugose fruit virus (ToBRFV) to generate resistant tomato plants

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Tomato (*Solanum lycopersicum L.*) is one of the world's most economically significant crops, yet its production is under severe threat from tomato brown rugose fruit virus (ToBRFV). This highly infectious virus has spread globally since its identification in 2015, overcoming traditional resistance genes (Tm-1, Tm-2, and Tm-22) and causing substantial yield losses. Current control strategies, such as quarantines and plant destruction, are insufficient due to the virus's stability and high transmission rates.

We will identify and characterize host susceptibility (S) genes necessary for ToBRFV infection in particular, tobamoviruses in general and unrelated viruses. We will use different methods to modulate the expression of identified S genes in tomato plants and assess the role of such S genes in resistance to ToBRFV and other viruses.

ManiVir: Host and vector manipulation following multi-infection of aphid transmitted viruses in sugar beet (*Beta vulgaris)*

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Sugar beet, which is an economically important crop in Europe, faces significant production challenges due to virus yellows, an aphid-transmitted disease caused by four different viruses: beet yellow virus (BYV, Closterovirus), beet mosaic virus (BtMV, Potyvirus), beet mild yellowing virus (BMYV, Polerovirus) and beet chlorosis virus (BChV, Polerovirus). These viruses, either individually or combined, can cause crop yield losses of up to 36% in fields (Hossain et al. 2020). Despite its economic impact, the interactions between these viruses and their collective influence on disease severity and crop productivity remains poorly understood. Our research focusses on exploring these plant-virus interactions and evaluating whether the combination of different viruses results in synergistic effects that amplify disease severity or antagonistic effects that reduce disease severity. While co-/double infections involving potyviruses are known to exacerbate disease severity and lead to severe yield losses (Wintermantel 2005), the consequences of superinfections - where one virus infects an already infected plant with other virus - remains unclear. Therefore, in this study, we investigated the effects of co- and superinfections of BtMV and BYV on sugar beet under controlled conditions to evaluate their effect based on their symptom development, plant parameters and beet weight. Our preliminary findings showed that co-infection with BtMV and BYV leads to significant reduction in plant shoot biomass and beet weight compared to single infections or healthy controls. On the other hand, superinfection of BtMV followed by BYV did not result in additional yield losses, even though there was a slight but not significant drop in plant height. These results suggest that co- and super infections may follow a different pathophysiological mechanisms within the plant. Overall, these insights into virus-virus interactions within the 'virus yellows' complex provide critical knowledge for understanding disease dynamics. And, may serve as a basis for developing targeted and effective management strategies to mitigate the impact of this virus yellows disease on sugar beet production.

References

Hossain R, Menzel W, Lachmann C, Varrelmann M, 2020. New insights into virus yellows distribution in Europe and effects of beet yellows virus, beet mild yellowing virus, and beet chlorosis virus on sugar beet yield following field inoculation. Plant Pathology, 70.

Wintermantel WM, 2005. Co-infection of Beet mosaic virus with Beet Yellowing Viruses Leads to Increased Symptom Expression on Sugar Beet. Plant disease, 89 (3), 325–331.

PV*Dive*: a sneak peek of a new online resource for plant virology

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Plant viruses are a diverse class of organisms that can cause disease in plants and as such pose a serious threat to agricultural crop production and food security. For advancements in the field, managing and exploiting vast amounts of data being generated by latest technologies is becoming increasingly important. However, publicly available data on plant viruses are at present not easily accessible; they are non-standardised, scattered across different resources, poorly interlinked and not quickly searchable by scientists or digital agents.

Here we present the first prototype of PV*Dive*, a new online resource aiming to address some of these challenges. In the initial stage, PV*Dive* will make the plant virology data associated with DSMZ's collection of plant viruses publicly available, i.e. information related to the origin of isolates, taxonomy, genome sequences and images of viral infections in different plant hosts. The information provided will be structured and linked to other data sources and databases, such as ICTV, GenBank, EPPO and others. An advanced search will enable good searchability of data by any of the data fields. Improved data accessibility will provide the basis for growing and enriching the database in later stages, for example by integrating other datasets, or by using AI tools to automatically extract information from other sources. It will also open doors for development of novel bioinformatics tools that will facilitate plant virology research.

Tobamovirus-equipped 3D matrices for biosensing in mini-columns and flow cells

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Tobacco mosaic virus (TMV) is a well-known tube-shaped RNA virus, with a genome consisting of 6395 nucleotides. With a length of 300 nm, an outer diameter of 18 nm, and an inner longitudinal channel of 4 nm width its outer surface is that of 2130 coat proteins (CPs), each of 17.5 kDa molecular weight. Their N- and C-termini are both exposed outside, offering accessible sites for genetic modifications, e.g., for the coupling of biomolecules such as enzymes. These characteristics render TMV particles attractive immobilization scaffolds for receptor and reporter units at immense surface densities. Our previous work has shown outstanding long-term shelf-life and reusability of TMV-displayed enzymes detecting the presence of different analytes, thereby imparting excellent properties to new types of biosensors (Koch, Poghossian et al. 2018).

The objective of ongoing research is to develop miniaturized columns and flow-through devices capable of a selective, robust and highly sensitive analyte detection via sensor enzymes installed on TMV coatings on appropriate matrix materials. Relevant targets include glucose, urea, acetoin, and antibiotics, for potential on-site applications in biomedicine, environmental and food monitoring. For this purpose, we had to **firstly** develop 3D matrices exposing TMV nanorods, which permit the flow-through of liquids. To this end, the binding abilities of different silica materials with TMV particles were investigated. Glass fibers exhibited a good binding capacity for TMVs in the presence of a polyelectrolyte interlayer. The **subsequent step** involves the preferably fast and easy attachment of sensor enzymes to the TMV nanorods. New types of engineered, systemically infectious TMV variants were developed, including such equipped with a Strep-tag II peptide at the C-terminus of every CP. These exhibit a direct biological binding capability for streptavidin (SA) conjugates and can thereby serve as a replacement for chemically biotinylated TMVcys and TMVlys applied as enzyme immobilization adapters in previous biosensor layouts (Geiger, Eber et al. 2013, Koch, Wabbel et al. 2015). As fully biological high surface-area nanocarriers, TMVStrepTag particles pave a novel route towards a sustainable, environmentally friendly and highly efficient immobilization approach for biomolecules. The concept was demonstrated using TMV-equipped mini-columns. **Currently,** TMV-enzyme combinations on glass fiber matrices are being examined in flow cells, as a starting point for the development of portable devices, loaded with advantageous 3D bio-affinity matrices for sensor enzymes. In first layouts of such microfluidic cells, the activities of streptavidin-horseradish peroxidase (SA-HRP) immobilized on TMVStrepTag coatings surpassed those of SA-HRP attached in the absence of TMV nano-scaffolds substantially. **In future work**, the presence of analytes in liquid samples passaging the flow-through system may not only be investigated through colorimetric quantification, but might also be achieved by electrochemical read-out, aiming at the fabrication of durable, reusable microfluidics-based point-of-care biosensors for relevant analytes.

References (see https://www.bio.uni-stuttgart.de/en/molbio/publications/ for a full list)

Geiger, F. C., F. J. Eber, S. Eiben, A. Mueller, H. Jeske, J. P. Spatz and C. Wege (2013). "TMV nanorods with programmed longitudinal domains of differently addressable coat proteins." Nanoscale 5(9): 3808-3816.

Koch, C., A. Poghossian, C. Wege and M. J. Schöning (2018). "TMV-Based Adapter Templates for Enhanced Enzyme Loading in Biosensor Applications." Methods Mol Biol 1776: 553-568.

Koch, C., K. Wabbel, F. J. Eber, P. Krolla-Sidenstein, C. Azucena, H. Gliemann, S. Eiben, F. Geiger and C. Wege (2015). "Modified TMV Particles as Beneficial Scaffolds to Present Sensor Enzymes." Front Plant Sci 6: 1137.

Untersuchung der Viruskrankheiten im Knoblauchanbau auf Mauritius

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Knoblauch ist ein essentieller Bestandteil der lokalen Küche der rund 1,3 Millionen Einwohner der Insel Mauritius im Indischen Ozean. Die Produktion ist jedoch in den letzten 40 Jahren von 325 t in 1980 auf rund 52 t in 2023 zurückgegangen. Die Flächenerträge sind in den letzten Jahren dramatisch eingebrochen, von 9,6t/ha im Jahr 2000 auf nur noch 4,4t/ha in 2023, was zu deutlich gestiegen Importen geführt hat. Für diesen Produktionsrückgang wird in erster Linie der Qualitätsverlust der einheimischen Knoblauchsorten verantwortlich gemacht. Ursächlich hierfür scheint die Akkumulation von unterschiedlichsten Viren im vegetativ vermehrten Pflanzgut über die letzten Jahrzehnte. Um Strategien entwickeln zu können, die nationale Produktion wieder zu steigern, soll in einem ersten Schritt eine globale Bestandsaufnahme der beteiligten Viren erfolgen. In Feldbegehungen 2023/24 wurden zwischen 50 und 70% symptomatische Pflanzen (u.a. Vergilbungen, Strichel, Mosaik, Blattrollen, Eintrocknen der Blattspitzen) in unterschiedlichen Anbaugebieten beobachtet, was das Ausmaß des Problems verdeutlicht. In serologischen Voruntersuchungen wurden die Allexiviren Garlic virus A und Garlic virus B nachgewiesen. Für eine umfassende Ermittlung des Viroms wurden 13 Blatt- und Knollenproben verschiedener Knoblauchlinien und Anbaugebiet in 3 Mischproben mittels Illumina Hochdurchsatzsequenzierung (HTS) untersucht. In den Proben konnten die Allexiviren garlic virus A, B, C, D und J nachgewiesen werden. Aus der Familie der *Betaflexiviridae* wurde das Carlavirus Garlic common latent virus mit verschiedenen Sequenzvarianten sowie weitere Contigs gefunden werden, die keiner bekannten Virusspezies zugeordnet werden können. Die größten Sequenzidentitäten wurden zu den neu vorgeschlagenen Virusspezies *Garlic yellow mosaic-associated virus* und *Garlic yellow curl virus* gefunden, für welche bereits angedacht wurde, ein neues Genus in der Familie *Betaflexiviridae* zu etablieren. Darüber hinaus konnten in den Proben die Potyviren Leek yellow stripe virus und Onion yellow dwarf virus sowie ein noch nicht bekanntes Polerovirus identifiziert werden. Die Ergebnisse sind abermals ein Beleg für die Vielfalt der in Knoblauch vorkommenden Viren. Die Untersuchungen auf Mauritius werden in den kommenden Jahren fortgeführt und auf weitere Regionen ausgedehnt. Darüber hinaus sollen die Erzeuger für die Notwendigkeit eines angemessenen Umgangs mit Virusinfektionen sensibilisiert werden, um den Ertrag und die Qualität zu steigern, mit dem Ziel die Abhängigkeit von Importen zu verringern.

The value of authenticated and characterized plant virus reference material: the case of potato virus Y in the collection at Leibniz Institute DSMZ

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Potato virus Y (PVY) is the most important viral pathogen threatening potato production worldwide. The biological diversity of PVY strains is known since the early 20th century, and an increasing number of strains has been classified and characterized over the years, revealing high genetic diversity and continuous evolution with emergence of recombinant strains. Such genetic variability is advantageous to the virus and enables PVY to survive in various conditions and hosts, also including tobacco, tomato and pepper, however, it poses a significant challenge for accurate detection and disease management, having foundation on precise diagnostics to control the trade of tubers and select healthy plant material for propagation. In this context, the availability of authenticated and characterized isolates represents a high-value source to provide reference material for the validation of serological and molecular diagnostic assays and is an important source of samples for proficiency tests. The Plant Virus Collection at Leibniz Institute DSMZ disposes currently of 46 PVY isolates of worldwide origin. A consistent number (35 accessions) has been fully characterized at molecular level by reconstruction of (nearly) complete genome sequences via high-throughput sequencing. The sequence analysis nicely shows the spread of the DSMZ isolates in the PVY phylogenetic tree, with accessions available for most of the so far defined clades (Lacomme et al., 2017; Della Bartola et al., 2020). The availability of such a comprehensive collection of PVY isolates is crucial for ensuring the accuracy and reliability of diagnostic tests. Further, these resources are of value for studies on PVY genetic diversity and evolution, and to enable the development of reliable diagnostic tools to safeguard potato production against this highly diverse virus.

Reference list

Della Bartola, M., Byrne, S., & Mullins, E. (2020). Characterization of potato virus Y isolates and assessment of nanopore sequencing to detect and genotype potato viruses. *Viruses*, *12*(4), 478.

Lacomme, C., Glais, L., Bellstedt, D. U., Dupuis, B., Karasev, A. V., Jacquot, E. (Eds.) (2017). Potato virus Y: biodiversity, pathogenicity, epidemiology and management (pp. 141-176). Basel, Switzerland. Springer International Publishing.

Green synthesis of silver nanoparticles from *Dioscorea alata* leaves and evaluation of their potential for virus elimination in yam tissue culture

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Yam (*Dioscorea* spp.) is an essential food crop in tropical and subtropical countries. Propagation of yam occurs vegetatively through tubers and this favors the accumulation of viruses. Green synthesis of nanoparticles has gained recent attention due to the demand for sustainable alternatives in the control of phytopathogens (dos Santos *et al.*, 2024). In this study, silver nanoparticles (AgNPs) were synthesized from different concentrations of aqueous extract of *Dioscorea alata* leaves and characterized for their physicochemical properties. The sample with the best characteristics was selected to evaluate the efficacy of AgNPs for virus elimination in yam (*D. cayennensis-rotundata*) plantlets infected with yam mosaic virus (YMV). The treatment of apical stems in *in vitro* tissue culture included the addition of AgNPs at a concentration of 25 µM to the medium, and a negative control. In parallel, two electrotherapy treatments, exposure of apical stems at 15mA current for 5 minutes and at 15V electric voltage for 15 minutes, were evaluated. Six plantlets were subjected to control treatment, thirteen plantlets to AgNPs treatment, ten to electrotherapy treatment at 15mA current, and seven to electrotherapy treatment at 15V electric voltage. Ninety days after treatment, total RNA was extracted from the plants grown in tissue culture (Bekesiova et al., 1999) and RT-PCR to detect YMV was performed. The results showed that YMV was detected in all plantlets grown in the negative control medium and in those subjected to electrotherapy at 15mA current, in six plants subjected to electrotherapy at 15V electric voltage and in only three plants grown in the medium treated with AgNPs. The preliminary results indicate that the treatment with AgNPs at 25 µM had a positive effect on YMV cleaning, with a more prominent effect than electrotherapy, therefore pointing to the green synthesis of AgNPs as a possible promising alternative for virus elimination in *Dioscorea* spp. plant tissues.

Reference List

Bekesiova I, Nap J P, Mlynarova L. 1999. Isolation of high-quality DNA and RNA from leaves of the carnivorous plant *Drosera rotundifolia*. *Plant Molecular Biology Reporter* **17**:269–277. https://doi.org/10.1023/A:1007627509824.

dos Santos O A L., dos Santos M S, Antunes Filho S, Backx B P. 2024. Nanotechnology for the control of plant pathogens and pests. *Plant Nano Biology* 100080. https://doi.org/10.1016/j.plana.2024.100080.

Detection and *In Vitro* Assessment of Viral Infections in Birch Trees (*Betula* spp.)

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Birch trees (*Betula* spp.) are increasingly threatened by viral infections. This study focuses on detecting viral infections in birch buds and explores symptom expression in *in vitro* plants. Birch leafroll decline is primarily caused by birch leaf roll-associated virus (*Badnavirus volubetulae*, BLRaV) (Rumbou et al. 2020; Vainio et al. 2024). Mixed infections of BLRaV with cherry leaf roll virus (*Nepovirus avii*, CLRV), birch carlavirus (*Carlavirus betulae*, BiCV), and birch idaeovirus (BIV) (Rumbou et al. 2020) may have synergistic effects and are associated with symptoms such as leaf deformation, chlorosis, and necrosis. These symptoms appear unevenly across branches, complicating diagnosis. Buds from infected trees were collected in January and February, sterilized, and cultured on Woody Plant Medium (WPM) and Murashige and Skoog Medium (MS) with varying hormone and sugar levels. RNA was extracted from single buds, as well as from mixed double and triple unopened buds, and from developed leaves of *in vitro* plants. Symptom expression in grown leaves was monitored across different media. We established an RT-PCR method for virus detection in buds, demonstrating that culturing buds as early as January or February provides sufficient leaf material for molecular analysis and visual symptom assessment. This approach offers a practical early-screening tool for producing virus-free plants for various applications. Our results show that overwintered buds developed primary leaves within 2–6 weeks, with symptom severity increasing in low-sugar media. However, these leaves ultimately perished without further shoot development. These findings highlight the influence of culture medium composition on viral symptom expression in birch propagation and underscore the importance of early detection for disease management. Further studies are needed to elucidate the molecular mechanisms underlying the relationship between sugar supply and virus-induced symptom development.

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Rumbou A, Candresse T, Marais A., Svanella-Dumas L, Landgraf M, von Bargen S, Büttner C, (2020): Unravelling the virome in birch: RNA-Seq reveals a complex of known and novel viruses. PLos ONE 15(6): e0221834, doi: https://doi.org/10.1371/journal.pone.0221834.

Vainio EJ, Rumbou A, Diez JJ, Büttner C (2024): Forest Tree Virome as a Source of Tree Diseases and Biological Control Agents. Current Forestry Reports, doi: https://doi.org/10.1007/s40725-024-00214-8.

Exploring the Molecular Mechanisms of Viral Co-Infection Impact on Aphid-Mediated Virus Transmission

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In this project, we will study the interactions in co-infection of sugar beet with BYV (family Closteroviridae) and BChV (family Solemoviridae). Both of them are restricted to the phloem. Their inoculation and transmission on plant host largely require vectors, like aphid, to feed on phloem tissue (companion cells and sieve tubes). However, the modes of interaction between these two viruses and aphids are very different: BYV is retained at the outer part of the feeding apparatus (likely in the cibarium), while BChV passes through the gut, reaches the hemolymph, and infects the salivary glands to be inoculated into a new host as part of the saliva. Different ways of interactions result in BChV transmitting throughout their lifespan, while BYV is lost after a few days or during molts. The modes of interactions between these two viruses and host plants remain unclear. Our previous results (Khechmar et al., 2024; thesis of Souheyla Khechmar, 2024) show a reduction in the transmission of BChV from plants co-infected with BVY, while BYV transmission is unaffected. This is neither due to a decreased viral accumulation nor altered aphid behavior on co-infected plants. At the cellular level, BChV is observed homogeneously throughout the cytosol in mono-infected plants, while BYV localizes in spherical inclusions. However, in co-infected plants, BYV co-localizes with BChV homogeneously throughout the cytosol, indicating a potential interaction between the viruses that may explain the decreased BChV transmission and viral redistribution.

The interactions among multiple viruses, host plants, and transmission vectors remain largely understudied, yet they could be crucial for advancing future virus and pest management strategies. This project aims to enhance our fundamental understanding of this pathosystem, shedding light for understanding viral cellular tropism and inspiring future pathologic control of plant viruses.

THE TOBAMOVIRUS SPHERE OF ORNAMENTALS ILLUSTRATED BY HOYA, A MEMBER OF THE APOCYNACEAE

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Background and Aims

Tobamoviruses with the type member tobacco mosaic virus (TMV) were seminal for plant virology. In general, these single stranded RNA viruses are highly contagious, replicating to eminent titers in infected tissues. Global trade, breeding as well as cultivation methods have contributed to their worldwide spread and have effected evolution of tobamoviruses that can cause serious economic losses in horticultural crop plants.

Methods

To study the interferences of Hoya tobamovirus 2 (HoTV2), with the host in single and mixed infections we developed a real time RT-PCR in *Nicotiana benthamiana* and employed high throughput sequencing for analyzes of small RNAs.

Results

Asymptomatic tobamoviruses have been isolated from *Hoya*, from *Hibiscus* and from *Brugmansia*. There are 200–300 species in the genus *Hoya* most of which are developing as epiphytes. Its topographical range comprises tropical and subtropical countries in Asia and Western Pacific. *Hoya* is known for its food and medicinal value in eastern cultures and became popular as indoor and outdoor plant due to its easy handling and spectacular appearances. Interestingly, symptomatic and asymptomatic tobamoviruses utilize *Hoya* as a host plant. Co-inoculation reduced TMV titers and inhibited systemic movement, whereas virus titer and movement stayed the same for HoTV2.

Conclusions

Small RNAs (sRNAs) serve as signaling molecules to regulate the host's defense pathways. We are currently analyzing the sRNA pattern in single and coinfections to learn more about the virus degradation and host regulation based on RNA interference during distinct tobamovirus infections.

Comparison of Partial RNA1 Sequences of Ash Shoestring-Associated Virus

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The role of viruses in forest ecosystems, particularly those affecting economically and ecologically significant tree species, has been largely underexplored due to previous technological limitations. However, next-generation sequencing (NGS) technologies have significantly enhanced our understanding of these viral threats (Rumbou et al., 2021; Büttner et al., 2023; Vainio et al., 2024). One such virus, ash shoestring-associated virus (ASaV), was first identified in common ash (*Fraxinus excelsior* L.) trees in Switzerland in 2016, exhibiting symptoms such as leaf mottling, chlorosis, leaf curling, and shoestring formation. ASaV has since been detected in flowering ash (*F. ornus* L.) (Gaskin et al., 2021). It belongs to the genus *Emaravirus* (order *Elliovirales*, family *Fimoviridae*) (Digiaro et al., 2024). The virus's genome consists of five single-stranded, negative-sense RNAs (RNA1-RNA5), each encoding a distinct protein: RNA-dependent RNA polymerase (RdRP, 268 kDa), glycoprotein precursor (GPP, 73 kDa), nucleocapsid protein (NP, 35 kDa), movement protein (MP, 41 kDa), and a 26 kDa protein of unknown function (P5) (Gaskin et al., 2021). Partial RdRP of ASaV was amplified in some isolates from different proveniences via RT-PCR using specific primers. The amplicons were cloned and sequenced. Sequence comparison revealed an identity of over 97% at the amino acid level among the studied isolates.

Literatur

Büttner C, Landgraf M, Colino H, von Bargen S, Bandte M (2023): Virus diseases of forest and urban trees. In Forest Microbiology; Asiegbu F and Kovalchuk A, Eds.; Academic Press, vol 3, pp.61-97. https://doi: 10.1016/B978-0-443-18694-3.00011-0

Digiaro M, Elbeaino T, Kubota K, Ochoa–Corona FM, von Bargen S (2024): ICTV Consortium. ICTV Virus Taxonomy Profile: Fimoviridae. *J. Gen. Virol.* 105(5). https://doi.org/ 10.1099/jgv.0.001943

Gaskin TR, Tischendorf M, Günther I, Rehanek M, Büttner C, von Bargen S (2021): Characterization of a Novel Emaravirus Affecting Ash Species (Fraxinus Spp.) in Europe. *Forests*, 12, 1–21. https://doi.org/10.3390/f12111574

Rumbou A, Vainio EJ, Büttner C (2021): Towards the Forest Virome: High–Throughput Sequencing Drastically Expands Our Understanding on Virosphere in Temperate Forest Ecosystems. *Microorganisms*, 9, 1730. https://doi.org/10.3390/microorganisms9081730

Vainio EJ, Rumbou A, Diez JJ, Büttner C (2024): Forest Tree Virome as a Source of Tree Diseases and Biological Control Agents. Curr. For. Rep. 10, 153–174. https://doi.org/10.1007/s40725-024-00214-8