20es RENCONTRES de Virologie Végétale

CAES du CNRS - **CENTRE PAUL-LANGEVIN**

AUSSOIS - Savoie - France

Du **19** au **23**

JANVIER 2025

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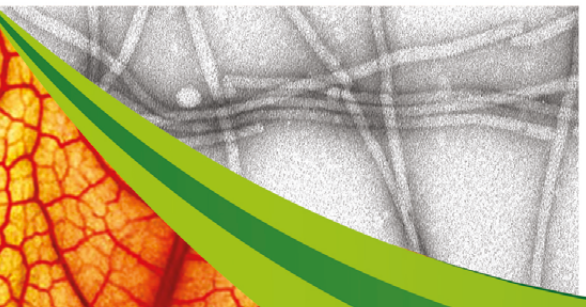

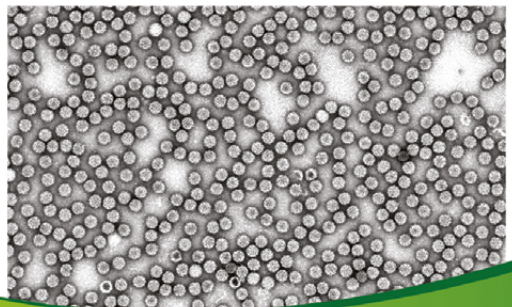

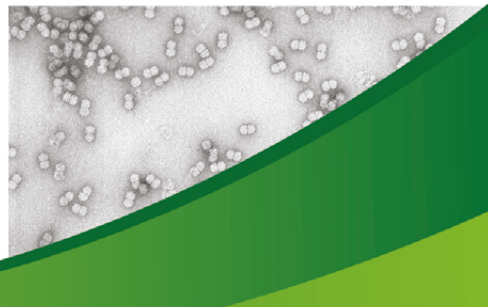

www.alphavisa.com/rvv/2025

BIENVENUE AUX 20es RENCONTRES DE VIROLOGIE VÉGÉTALE

Cher(e)s ami(e)s et collègues,

Les **Rencontres de Virologie Végétale (RVV)** ont lieu tous les deux ans, depuis 1987. Elles réunissent tous les acteurs de la Virologie Végétale en France (doctorants, enseignants, techniciens, ingénieurs et chercheurs des organismes publics et privés) et des scientifiques de laboratoire étrangers. Grâce à la participation de plus de 150 personnes, elles permettent de faire état des résultats récents et de l'évolution des recherches.

Ces rencontres couvrent des disciplines variées de la virologie végétale, et font aussi appel à des intervenants spécialisés dans des domaines de virologie animale ou environnementale qui ont fait l'objet d'avancées récentes importantes.

Les RVV allient excellence scientifique et convivialité, et représentent des opportunités d'échanges entre les différents acteurs de la recherche en virologie. Ce colloque favorise en particulier la rencontre entre les jeunes chercheurs et ceux plus confirmés. Il est à l'origine de nombreuses collaborations entre des laboratoires maitrisant des disciplines très diverses (biologistes moléculaires, généticiens, entomologistes, épidémiologistes, …).

Le Comité d'Organisation est très heureux et impatient de vous accueillir du **19 au 23 janvier 2025 au Centre Paul-Langevin à Aussois** pour la 20e édition des RVV.

N'oubliez pas de noter et bloquer dès à présent la date du colloque dans vos agendas !

Le Comité d'Organisation

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LES COMITÉS

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- **Stéphane BLANC** - UMR PHIM, INRAE, Montpellier, France
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- **Manuella Van MUNSTER** - UMR PHIM, INRAE, Montpellier, France

COMITÉ SCIENTIFIQUE

- **Stéphane BLANC** - UMR PHIM, INRAE, Montpellier, France
- **Justine CHARON** - UMR BFP, INRAE, Bordeaux, France
- **Sébastien MASSART** - Université de Liège, Gembloux, Belgique
- **Loup RIMBAUD** - Unité de Pathologie Végétale, INRAE, Avignon, France
- **Anne SICARD** - UMR SVQV, INRAE, Colmar, France
- **Lucie TAMISIER** - Unité GAFL, INRAE, Avignon, France
- **Marilyne UZEST** - UMR PHIM, INRAE, Montpellier, France
- **Manuella Van MUNSTER** - UMR PHIM, INRAE, Montpellier, France

Secrétariat administratif

Alpha Visa Congrès / RVV 2025
624 rue des Grèzes
34070 Montpellier - France
Tél. : +33 4 67 03 03 00
rvv-2025@alphavisa.com
https://www.alphavisa.com/rvv/2025

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NOS PARTENAIRES

INRAE - DPT SPE

Institut national de recherche pour l'agriculture, l'alimentation et l'environnement
Département Santé des Plantes et Environnement
BP 167 - 06903 Sophia Antipolis - France
Tél : +33 (0) 4 92 38 64 93
https://www.inrae.fr/

PÔLE DE RECHERCHE AEB

Agriculture-Environnement-Biodiversité
Université de Montpellier
163 rue Auguste Broussonnet
34090 Montpellier- France
https://www.umontpellier.fr/recherche/unites-de-recherche/pole-aeb-presentation

SFP

Société Française de Phytopathologie (Association loi 1901)
BUEE Marc - Secrétaire Général
UMR 1136 Interactions Arbres-Micoorganismes (IAM), INRAE
54280 Champenoux - France
marc.buee@inrae.fr
https://www.sfp-asso.org/

SFV

Société Française de Virologie (Association loi 1901)
Institut Pasteur
25 rue du Docteur Roux
75015 Paris - France
https://sfv-virologie.org/

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RÉSUMÉS DES COMMUNICATIONS AFFICHÉES

PROGRAMME - DIMANCHE 19 JANVIER

17:00 **Accueil des participants au Centre Paul-Langevin**

Mise en place des posters

19:15-20:15 **Apéritif de bienvenue**

20:15 **Dîner**

K Orateur-trice invité.e / *Keynote speaker*
C Évolution virale / *Virus evolution*
C Variabilité/diversité des virus & épidémiologie / *Virus variability/diversity & epidemiology*
C Résistance & silencing / *Resistance & silencing*
C Interaction virus/cellule & mouvement / *Virus-cell interaction & within plant movement*
C Interaction virus-vecteur / *Virus-vector interaction*

PROGRAMME - LUNDI 20 JANVIER

08:30-08:45 **Mot d'introduction au Colloque**

Par le Comité d'Organisation

08:45-10:15 **SESSION 1**

Modérateur : Philippe ROUMAGNAC

08:45-09:30 **Keynote 1**

- K-01 **- Anne-Claire BAUDOUX** - *CNRS, Station Biologique de Roscoff, Roscoff, France*
  Ecological implications of phytoplankton viruses in the ocean

09:30-10:15 **Communications orales**

09:30-09:45
- C-01 **- Armelle MARAIS**
  Vers une vision globale du virome des blés français

09:45-10:00
- C-02 **- Amélie JANZAM**
  Viral non-coding RNA resistant to exoribonuclease: synergic actor of RNA silencing suppression and promoter of viral long-distance movement

10:00-10:15
- C-03 **- Alexandre ROUDAUT**
  Molecular basis of resistance to viruses at the common bean NLRs I cluster

10:15-10:45 **Pause café - Posters**

10:45-12:30 **SESSION 2**

Modératrice : Cica URBINO

10:45-11:30 **Keynote 2**

- K-02 **- Rosa LOZANO-DURÁN** - *ZMBP, Universität Tübingen, Tübingen, Allemagne*
  Hostile takeover - elucidating the subversion of the plant by geminiviruses

11:30-12:30 **Communications orales**

11:30-11:45
- C-04 **- Emanuela NORIS**
  Insights into the role of the C4 protein of the geminivirus TYLCSV in transgenic tomato plants

... /...

Retour sommaire

11:45-12:00 • C-05 - **Charlotte TOLLENAERE**
Co-circulation of multiple pathogens and population-scale consequences

12:00-12:15 • C-06 - **Margaux GENARD**
How the first *Polymyxa graminis* f. sp. colombiana genome could help unraveling *Polymyxa graminis* interactions with its host plants and the *Rice stripe necrosis virus*?

12:15-12:30 • C-07 - **Lucas SCHALCK**
Exploring the intriguing role of the TuYV's P3a protein

12:30 **Déjeuner**

Après-midi libre

17:15-18:30 **SESSION 3**

Modératrice : Lucie TAMISIER

17:15-18:15 **Communications orales**

17:15-17:30 • C-08 - **Jean-Luc GALLOIS**
eIF4E1 precision editing provides a solution to the trade-off between the plant development and potyvirus resistance albeit with a low durability

17:30-17:45 • C-09 - **Norman DAURELLE**
Estimation des nombres de reproduction de base et effectif de la sharka

17:45-18:00 • C-10 - **Lola CHATEAU**
Relations entre virulence, charge virale et transmission du potato virus Y (genre *Potyvirus* ; famille Potyviridae)

18:00-18:15 • C-11 - **Fanny LELEUX**
Does high virus prevalence in wild plants mean beneficial co-evolution? A case study on banana mild mosaic virus

18:15-18:30 **Flash talk posters**

Présentation Flash des posters proposés par les étudiants

- P-02 - **Seydou SAWADOGO**
Improving Cassava Mosaic Disease detection through Oxford Nanopore Technologies Approach on cassava leave in Burkina Faso
- P-03 - **Léna JAMBOU**
Lipid droplets: New actors of the plant virus infection
- P-15 - **Léo BLONDET**
Bdv2 resistance gene against barley yellow dwarf disease in wheat
- P-34 - **Alice MERCEY**
The aphid-transmission of nanoviruses involves an atypical Helper Component
- P-38 - **Geoffroy MILKOFF**
Strategies for developing new genetic resistances to viruses based on the susceptibility factors phosphoglycerate kinases
- P-45 - **Gaël REVERT**
Caractérisation partielle du virome d'*Ipomoea batatas (Convolvulaceæ)* et de *Dioscorea sp. (Dioscoreaceæ)* dans le Grand-Ouest français

18:30-20:00 **Session posters**

20:00 **Dîner**

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PROGRAMME - MARDI 21 JANVIER

08:30-10:15 **SESSION 4**

Modérateur : Thierry CANDRESSE

08:30-09:15 **Keynote 3**

- **K-03 - Marco FORGIA** - *CNR, IPSP, Turin, Italie*
  Unveiling the molecular mechanisms and the biological effects associated with infectious agents with viroid-like properties (ribozycirculome) in fungi

09:15-10:15 **Communications orales**

09:15-09:30
- **C-12 - François MACLOT**
  Deciphering the complex ecology of plant and mycoviruses in wild grasses by analyzing the virome associated with individual plants

09:30-09:45
- **C-13 - Laura DI PIETRO**
  Mapping cell-type specific localization of plant viruses and effectors in aphid salivary glands with advanced FISH techniques

09:45-10:00
- **C-14 - Gaël THÉBAUD**
  Canine detection of plant viruses: a proof-of-concept with the plum pox virus

10:00-10:15
- **C-15 - Lavena Van CRANENBROECK**
  Exploring a new territory for grapevine viruses: first outputs from winemaker interviews and virome survey in Belgium

10:15-10:45 **Pause café - Posters**

10:45-12:30 **SESSION 5**

Modérateur : Quentin CHESNAIS

10:45-11:30 **Keynote 4**

- **K-04 - Pauline EZANNO** - *INRAE, ONIRIS, VetAgroBio, Nantes, France*
  Multiscale modelling of arthropod-borne virus transmission dynamics

11:30-12:30 **Communications orales**

11:30-11:45
- **C-16 - Loup RIMBAUD**
  The spicy dissemination of cucumber mosaic virus in Espelette

11:45-12:00
- **C-17 - Fani GOUSI**
  A virus-induced gene silencing (VIGS) approach to study plant-geminivirus-insect vector interactions and virus transmission

12:00-12:15
- **C-18 - Damien Richard**
  Genomic insights into the global emergence of the phytopathogenic Maize yellow mosaic virus

12:15-12:30
- **C-19 - Nikolay SIMANKOV**
  Integrative Proteomics for Predicting Biological Properties of Emerging Plant Viruses: A Genomics-Driven Machine Learning Framework

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12:30 **Déjeuner**

Après-midi libre

17:15-18:30 **SESSION 6**

Modérateur : Loup RIMBAUD

Communications orales

17:15-17:30 • **C-20 - Pierre MUSTIN**
Get to know each other: Considering the genetic diversity of grapevine fanleaf virus at a local scale prior to a cross-protection trial in the field

17:30-17:45 • **C-21 - Emma-Louise JAFFRÉ**
Phenotypic characterization of the resistance observed in *Oryza glaberrima* accession against RSNV

17:45-18:00 • **C-22 - Prune LACÔTE-POPOVIC**
Tissue-Specific Variations in the Frequency Distribution of a Multipartite Virus Genomic Components

18:00-18:15 • **C-23 - Elise LEPAGE**
Cucumber mosaic virus degrades pepper fruit production, marketability and organoleptic quality, with isolate-specific effects

18:15-18:30 • **C-24 - Souheyla KHECHMAR**
Synergistic interactions between the beet mosaic potyvirus and the beet yellows closterovirus decrease transmission of the closterovirus

18:30-20:00 **Session posters**

20:00 **Dîner**

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PROGRAMME - MERCREDI 22 JANVIER

08:30-10:15 **SESSION 7**

Modératrice : Sylvie GERMAN-RETANA

08:30-09:15 **Keynote 5**

- **K-05 - Julien POMPON** - *IRD, MIVEGEC, Montpellier, France*
  Mosquito salivary transmission enhancerS

09:15-10:15 **Communications orales**

09:15-09:30
- **C-25 - Marguerite BATSALE**
  Viral manipulation of lipid droplets: insights from turnip mosaic virus infection in plants

09:30-09:45
- **C-26 - Thomas ARMAND**
  Characterization of host quality of non-crop Poaceae species for wheat dwarf virus and *Psammotettix alienus*

09:45-10:00
- **C-27 - Valentin GUYOT**
  Transcriptome and small RNAome profiling reveals the molecular mechanisms underlying plant-nanovirus-aphid vector interactions

10:00-10:15
- **C-28 - Dalia DJABOUB**
  OptiCQua: Optimizing the performance of sanitary diagnosis of *Vitis* spp. submitted to certification and quarantine schemes

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10:15-10:45 **Pause café - Posters**

10:45-12:30 **SESSION 8**

Modérateur : Yannis MICHALAKIS

10:45-11:30 **Keynote 6**

- **K-06 - Israël PAGAN** - *Universidad politecnica de Madrid, Centro de biotecnologia y genomica de plantas, Madrid, Espagne*
  Plant-virus co-evolution during adaptation to the transmission mode

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11:30-12:30 **Communications orales**

11:30-11:45
- **C-29 - Julien HENNEQUART**
  Genetic architecture of Tobacco mosaic virus tolerance in *Arabidopsis thaliana*

11:45-12:00
- **C-30 - Atiwich PATTHAMAPORNSIRIKUL**
  Does multiple infection of tomato leaf curl New Delhi virus (ToLCNDV) and watermelon mosaic virus (WMV) cause a more severe disease in melon?

12:00-12:15
- **C-31 - Auguste RAJOT**
  Making use of an axillary component from the multipartite nanovirus for virus-induced silencing of insect vector genes

12:15-12:30
- **C-32 - Wassim RHALLOUSSI**
  Datamining public databases in search of grapevine viruses

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12:30 **Déjeuner**

Après-midi libre

17:15-18:30 **SESSION 9**

Modératrice : Anne SICARD

Communications orales

17:15-17:30 • C-33 **- Estel Pakyendou NAME**
Unexpected virus reservoirs in vegetatively propagated plants: A case study of sweetpotato

17:30-17:45 • C-34 **- Anita KALTAK**
MED18: a bridge for cauliflower mosaic virus mRNAs export

17:45-18:00 • C-35 **- Maximilian SCHUGHART**
Identifying the impact of insecticide resistant grain aphids (Sitobion avenae) on barley yellow dwarf virus epidemiology in Ireland

18:00-18:15 • C-36 **- Heemee Devi BUNWAREE**
Development of polerovirus-induced gene silencing for rapid and visual screening in sugar beets

18:15-18:30 • C-37 **- Pierre HELLIN**
Discovery and biological characterization of a novel emaravirus infecting blackberry in Belgium

18:30-20:00 **Session posters**

20:00 **Dîner**

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PROGRAMME - JEUDI 23 JANVIER

09:15-10:15 **SESSION 10**

Modératrice : Corinne KEICHINGER

Communications orales

09:15-09:30 • C-38 - **Nils POULICARD**
Dispersion and evolutionary history of rice yellow mottle virus in Africa: tales of rice and men

09:30-09:45 • C-39 - **Cica URBINO**
Role of a short non coding viral sequence in bypassing crossprotection in tomato infecting begomoviruses

09:45-10:00 • C-40 - **Véronique BRAULT**
Insights into mature plant resistance in sugar beet and inhibition of virus transmission by aphids

10:00-10:15 • C-41 - **Judith HIRSCH**
Uncovering the genetic basis of quantitative resistance to cucumber mosaic virus in Capsicum annuum using genome-wide association coupled with high-throughput image analysis

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10:15-10:45 **Pause café - Posters**

10:45-11:45 **SESSION 11**

Modérateur : Stefano COLELLA

Communications orales

10:45-11:00 • C-42 - **Emeline TEYSSIER**
Plasma membrane-localized lipids are involved in plant response during infection by *Potexviruses*

11:00-11:15 • C-43 - **Khalid AMARI**
Exogenous application of dsRNA to control plant pathogens and pests: it works! Now what?

11:15-11:30 • C-44 - **Benoît MOURY**
Effect of mutations in intrinsically-disordered or ordered regions of potato virus Y (PVY ; genus *Potyvirus* ; family Potyviridae) VPg on virus fitness and adaptability

11:30-11:45 • C-45 - **Thierry CANDRESSE**
À la recherche des réservoirs des virus responsables des jaunisses de la betterave

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11:45-12:15 **Remise des Prix & Bilan du Colloque**

Par le Comité d'Organisation

12:15 **Déjeuner & Départ**

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RÉSUMÉS DES COMMUNICATIONS

LUNDI 20 JANVIER

K-01

Ecological implications of phytoplankton viruses in the ocean

Nathalie Simon[1], David Demory[2], Laure Arsenieff[1], Emmanuelle Jaouen[1], Pauline Nogaret[1], Florence Le Gall[1], Estelle Bigeard[1], Marie Walde[1], Pei Ge[1], Chiara Fiorile[1], Fabienne Rigaud-Jalabert[3], Martin Gachenot[3], Christophe Six[1], Dominique Marie[1], Sophie Lepanse[3], Anne-Claire Baudoux[1] (acbaudoux@sb-roscoff.fr)

[1] *Sorbonne Université, CNRS, UMR7144 Adaptation et Diversité en Milieu Marin (AD2M), Station Biologique de Roscoff, Roscoff, France*
[2] *Sorbonne Université, CNRS, USR 3579 Laboratoire de Biodiversité et Biotechnologies Microbiennes (LBBM), Observatoire Océanologique, Banyuls-sur-Mer, France*
[3] *Sorbonne Université, CNRS, Fédération de recherche 2424 (FR2424), Station Biologique de Roscoff, Roscoff, France*

Although it represents only a tiny fraction (1%) of photosynthetic biomass, marine phytoplankton contributes nearly half of the planet's primary production. The carbon fixed by phytoplankton forms the foundation of food webs. When phytoplankton dies or is consumed by zooplankton, part of this carbon is also exported to the ocean floor, where it can be sequestered for millennia. This export, known as the biological carbon pump, directly influences the planet's climate. Our research aims to understand how viruses regulate the structure and functions of these photosynthetic microorganisms in the ocean. In this presentation, we will illustrate, through field, experimental, and theoretical studies, the importance of viruses in phytoplankton mortality, the regulation of these interactions in a rapidly changing ocean, and their implications in major biogeochemical cycles, particularly by modulating the metabolism of the organisms they infect.

Keywords: Viruses - Phytoplankton - Ocean - Ecology - Biogeochemistry.

Retour sommaire

C-01

Vers une vision globale du virome des blés français

Armelle Marais[1] (armelle.marais-colombel@inrae.fr), Thierry Candresse[2], Bernard Bergey[2], Chantal Faure[2], Laurence Svanella-Dumas[2], Lionel Lebreton[3], Cindy Vitry[4]

[1] *BFP, INRAE, Villenave-d'Ornon, France*
[2] *INRAE, Villenave-d'Ornon, France*
[3] *INRAE, Le Rheu, France*
[4] *Arvalis, Boigneville, France*

Le projet DEEP IMPACT vise à concevoir des solutions agroécologiques basées sur l'exploitation de la diversité du microbiote pour accroître la résistance des plantes aux bioagresseurs. Ainsi, le virome du blé a été caractérisé durant deux ans dans 100 parcelles localisées dans trois régions françaises aux conditions pédo-climatiques contrastées, et représentant une diversité de pratiques agricoles. Des blés ont été collectés indépendamment de symptômes visuels aux printemps 2022 et 2023, et soumis à une analyse métagénomique virale dsRNA (48 plantes poolées/parcelle).

Les résultats montrent un virome du blé constitué non seulement de virus connus pour infecter le blé, mais aussi d'un grand nombre de mycovirus. Trois nouveaux virus, peu prévalents, ont également été caractérisés : un furovirus, un pomovirus, un polérovirus. Parmi les phytovirus, les virus BYDV-PAS et BYDV-PAV sont majoritairement rencontrés, et de loin les plus prévalents parmi les membres du complexe d'espèces B/CYDV associé à la jaunisse nanisante, le BYDV-GAV/MAV et le polérovirus CYDV-RPS étant très sporadiquement détectés. Ces résultats sont cohérents avec ceux du projet CASDAR ViroCap, dans lequel 69 plants de blé présentant des symptômes de jaunisse nanisante et provenant de 29 départements ont été analysés par RNAseq, montrant à nouveau une forte dominance des BYDV-PAS et -PAV parmi les virus du complexe B/CYDV.

De façon plus globale, les résultats de DEEP IMPACT montrent une structuration régionale du virome, avec des différences très marquées notamment pour la présence des BYDV-PAS et -PAV. Une dynamique temporelle est également observée avec une variation de la prévalence de ces virus entre 2022 et 2023. Les analyses en cours permettront de confronter la composition du virome (phytovirome et mycovirome) de chaque parcelle à des modèles statistiques corrélatifs prenant en compte leurs caractéristiques biotiques et environnementales dans une vision holistique du microbiome et de sa contribution à la santé des cultures.

Mots clés : Virome - Blé - JNO - BYDV - Holobionte.

Retour sommaire

C-02

Viral non-coding RNA resistant to exoribonuclease: synergic actor of RNA silencing suppression and promoter of viral long-distance movement

Amélie Janzam (amelie.janzam@etu.unistra.fr), David Gilmer

UPR2357, Institut de Biologie moléculaire des plantes, Strasbourg, France

Beet necrotic yellow vein virus (BNYVV), responsible for sugar beet rhizomania, is a multipartite virus with four to five positive-strand RNA molecules, each encapsidated into helicoidal individual particles. For its long-distance movement in beet (*Beta macrocarpa*), BNYVV requires its coat protein (CP), three movement proteins, the RNA2 encoded viral suppressor of RNA silencing (VSR) and the accumulation of a viral non-coding RNA (vncRNA) acting synergistically on RNA silencing suppression. vncRNA is produced as a result of partial degradation of BNYVV genomic RNA3 by 5'-3' exoribonuclease (XRN4) which stalls thanks to the conserved and structured "coremin" motif. Furthermore, a nuclear localization of vncRNA3 is observed but the role behind this localization remains unknown. In this context, our research aims to characterize the ncRNA3 interactome to elucidate its function in the BNYVV viral cycle. To achieve this, we tested three different RNA pull-down methods to identify proteins binding specifically to ncRNA3. We compared wild type and an RNA3 mutant unable to produce vncRNA. First, we determined which of the three methods was optimal. We then identified proteins that are specifically enriched for ncRNA3 binding.
Our findings evidenced an enrichment of nuclear proteins that could be linked to the nuclear localization of ncRNA3, suggesting a possible role in modulating the plant transcriptome to support BNYVV infection. However, no protein known to be directly involved in RNA silencing was enriched, leaving the mechanism by which ncRNA3 exerts its VSR function still unclear.
Further validation of these interactants will require additional techniques, such as immunoprecipitation or yeast three-hybrid assay.

Mots clés : BNYVV - Viral non-coding RNA - Viral suppressor of RNA silencing - RNA pull-down - Interactome.

C-03

Molecular basis of resistance to viruses at the common bean NLRs *I* cluster

Alexandre Roudaut (alexandre.roudaut@universite-paris-saclay.fr)

IPS2, Université-Paris-Saclay, Gif-sur-Yvette, France

Common bean (*Phaseolus vulgaris*) is the most important grain legume for human consumption in the world. Many resistance genes, organized in subtelomeric clusters, have been identified in the common bean genome. The *I* cluster of TIR-NLRs (TNLs), located at one end of chromosome 2, holds genes of resistance against various pathogens, including the eponymous *I* gene of resistance to potyviruses including BCMV (Bean common mosaic virus), BCMNV (Bean common mosaic necrosis virus) and potentially nine other potyviruses, and the *R-BPMV* gene of resistance to a comovirus, BPMV (Bean Pod Mottle Virus). The *I* gene enables Extreme Resistance-mediated response to most BCMV strains, but triggers systemic HR (Hypersensitive Response) (a lethal resistance response) in plants infected by BCMNV. While the molecular basis of the *R-BPMV* gene remains unknown, recently, the TNL corresponding to the *I* gene was identified thanks to two independent mutants. In order to determine which protein of BCMNV corresponds to the avirulence factor (Avr) recognized by the *I* gene product to trigger HR, we performed an *Agrobacterium*-mediated transient expression assay of each of the 11 BCMNV proteins in *II* and *ii* common bean leaves. This allowed the identification of the NIa-Pro protease as the Avr protein from BCMNV which triggers HR only in *II* genotypes. A similar experimental design was conducted to identify the BPMV Avr recognized by *R-BPMV*; however, none of the 9 BPMV proteins trigger HR in transient expression in an *R-BPMV* genotype. We hypothesize that a BPMV polyprotein precursor rather than an individual protein might be recognized to trigger HR.

Mots clés : Potyvirus - Comovirus - Common bean - TIR-NLR - Hypersensitive Response.

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K-02

Hostile takeover – elucidating the subversion of the plant by geminiviruses

Rosa Lozano-Durán (rosa.lozano-duran@zmbp.uni-tuebingen.de)

Plant Biochemistry Dept., Center for Plant Molecular Biology (ZMBP), University of Tübingen, Germany

Viruses, as obligate intracellular parasites, need to subvert the host cell in order to enable their replication and efficient spread. Due to strict restrictions in genome size, viruses commonly produce a limited number of proteins; this is the case of geminiviruses, plant viruses with circular single-stranded (ss)DNA genomes that are believed to contain only 4-8 translated open reading frames. Strikingly, despite their limited armoury, geminiviruses are able to establish an infection, overcoming plant defence, dramatically altering plant development and physiology, and ultimately causing devastating diseases to crops worldwide. How these viruses successfully invade and manipulate their plant hosts by deploying only a handful of proteins is a long-standing, fascinating question. In our group, we are interested in understanding how geminiviruses co-opt the plant cell and lead to disease, for which we use a combination of approaches, including molecular biology, cell biology, and genetics. Our results have shed light onto the molecular mechanisms underlying the replication of viral DNA, plant anti-viral defence and geminiviral counter-defence, and symptom development, and hint at novel virulence strategies potentially employed by geminiviruses to maximize their coding capacity and their impact on the host cell. We expect that our work will contribute to a deeper understanding of the infection process, which may in turn pave the way to the design of effective and sustainable anti-viral strategies and assist breeding programs to obtain virus-resistant plants.

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C-04

Insights into the role of the C4 protein of the geminivirus TYLCSV in transgenic tomato plants

Emanuela Noris[1] (emanuela.noris@ipsp.cnr.it), Silvia Rotunno[1], Camilla Sacco Botto[1], Laura Miozzi[1], Marco Catoni[2]

[1] *Institute for Sustainable Plant Protection, CNR, Torino, Italie*
[2] *School of Biosciences, University of Birmingham, Birmingham, United Kingdom*

We previously showed that Tomato yellow leaf curl Sardinia virus (TYLCSV), a begomovirus belonging to the *Geminiviridae* family, confers enhanced tolerance to drought in tomato plants, possibly thanks to the intervention of the virus-encoded C4 protein, a small and highly variable protein with multifunctional roles (1). Transgenic tomato plants overexpressing the TYLCSV C4 protein showing morphological defects are resistant to drought (2) and fungal attack (3). To define the molecular basis potentially involved in the phenotype of C4 plants, we carried out an RNA-Seq analysis comparing plants overexpressing C4 with wild-type individuals. Differential expression analysis, followed by Gene Ontology and KEGG pathway enrichment analysis revealed the impact of C4 protein on the metabolism of nucleotides, starch, glucose, cell wall components, fatty acids, and plant hormones and on pathogen interaction. By RT-qPCR, we confirmed that key genes known to be involved in cell wall-related pathways are deregulated. This work contributes to shed light on the intricate interplay between geminiviruses and tomato plants under abiotic and biotic stresses.

Mots clés : Geminivirus - Transcriptome - Plant cell wall - Plant pathogen interaction.

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C-05

Co-circulation of multiple pathogens and population-scale consequences

Charlotte Tollenaere[1] (charlotte.tollenaere@ird.fr), Estelle Billard[1], Abalo Itolou Kassankogno[2], Martine Bangratz[1], Nils Poulicard[1], Phonsiri Saengram[1], Kader Abdoul Guigma[2], Aurore Comte[1], Eugénie Hébrard[1]

[1] *UMR PHIM, IRD, Montpellier, France*
[2] *Laboratoire de Phytopathologie, INERA, Bobo-Dioulasso, Burkina Faso*

Multiple infections, or 'co-infections', occur when a single host is infected by different pathogen species, or genotypes. This is known to affect symptom expression and pathogen multiplication in many pathosystems. However, the population-scale consequences remain poorly explored.
Rice grown in Burkina Faso is affected by multiple pests, of which the rice yellow mottle virus (RYMV) and the bacteria *Xanthomonas oryzae* (*Xo*) are known to interact in experimental co-infections. In addition, several lineages of the RYMV co-circulate locally, and were evidenced as mixed infections. In this project, we aimed to investigate the evolutionary consequences of multiple infections (both within and between species) and their role in maintaining viral genetic diversity, for the RYMV in Burkina Faso.
To this end, a stratified random sampling was performed in two sites, during two consecutive years (2021 and 2022). Molecular detection applied jointly to RYMV and *Xo* revealed a much higher frequency of samples co-infected by the two pathogens, than inferred from the observations of the two types of specific symptoms on the same plants. We then characterised the genetic diversity of RYMV in collected samples revealing a locally high diversity within fields, and significant structuration between the two sites. In addition, we found some evidence for an association of some RYMV lineages with the presence of *Xo,* suggesting reciprocal influence of co-infecting pathogens on the genetic structuration of other pathogen species. Further research could confirm such consequences of multiple infections on the evolution of viral populations.

Mots clés : Co-infections - Mixed infections - Genetic diversity - Africa - Rice.

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C-06

How the first *Polymyxa graminis* f. sp. *colombiana* genome could help unraveling *Polymyxa graminis* interactions with its host plants and the *Rice stripe necrosis virus*?

Margaux Genard[1] (margaux.genard@uclouvain.be), Claude Bragard[1], Laurence Albar[2], Eugénie Hébrard[2], Anne Legrève[1]

[1] *ELI/ELIM/SAVE, UCLouvain, Louvain-la-Neuve, Belgique*
[2] *PHIM/VICITA, IRD, Montpellier, France*

The *Rice stripe necrosis virus* (RSNV) is a Benyvirus spread across South America and re-emerging in West Africa, causing damages to rice crops [1,2]. It is transmitted by the plasmodiophorid *Polymyxa graminis* f. sp. *colombiana* (Pgcol), a rice root obligate endoparasite. However, the extent of host ranges for both the virus and Pgcol is still largely unknown. This presentation aims to unravel the host range of RSNV and its vector, examining whether they are restricted to rice or capable of infecting other cereals. Host range assays in controlled conditions for the RSNV and its vector on various cereals and sugar beet suggest larger host ranges than previously reported. Moreover, detection of RSNV without its vector on sorghum indicates that the penetration of the vector in root cells without further development could be sufficient for RSNV transmission, as previously reported for the *Peanut clump virus* transmitted by *Polymyxa graminis* f. sp. *tropicalis* [3]. Comparison of Pgcol incubation and multiplication rates, phenotype and its colonization capacity on different hosts will allow the characterization of specificity levels between Pgcol and its hosts. Additionally, the first genome sequencing of Pgcol will be presented, uncovering conserved genomic regions shared with *Polymyxa betae* that could be linked to the transmission of benyviruses. This will provide a foundation for understanding the molecular mechanisms behind *Polymyxa*-benyviruses interactions. Finally, a review of current phylogeny of *Polymyxa* species, including new sequences from isolates from Europe, North America, South America, and Africa and several new genes offers valuable insights into the evolution and geographic distribution of this genus of plant roots endoparasites.

Mots clés : Polymyxa graminis - RSNV - Host range - Transmission - Genome.

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C-07

Exploring the intriguing role of the TuYV's P3a protein

Lucas Schalck (lucas.schalck2@etu.unistra.fr), Véronique Ziegler-Graff

CNRS (UPR-23 57), Institut de Biologie moléculaire des plantes, Strasbourg, France

Turnip yellows virus (TuYV) is a plant virus responsible for significant yield losses in rapeseed crops worldwide. Like other members of the *Polerovirus* genus, it has atypical features, such as a phloem-restricted tropism and a range of translation mechanisms to express its proteins. Despite being identified and studied for several decades, researchers still face challenges in unraveling the molecular mechanisms involved in viral replication and movement. Ten years ago, we discovered the P3a, a 45 amino-acid-long polypeptide expressed through a small, non-canonical ORF, which is essential for the virus's long-distance movement (Smirnova *et al*., 2015).
Building on this work, my PhD project aims to investigate the extent to which P3a is involved in the movement process of the TuYV, stressing on the protein-protein interaction's aspect. To achieve that, the main strategy was to identify cellular/viral partners of recombinant P3a proteins through proteomics experiments using two model plants (*N. benthamiana* and *A. thaliana*), while assessing their subcellular localization in confocal microscopy using different cell markers. These core experiments were followed by additional molecular and microscopy-based techniques (reversed co-IPs, FRET-FLIM and BiFC) to validate the interaction between the P3a and some statistically enriched proteins, including proteins involved in endocytosis and cell trafficking. In addition, their hypothetical role during TuYV's infection is being explored *via*agro-infection of knockout plants. Collectively, these preliminary results could pave the way to progressively understand how the intriguing P3a works.

Mots clés : Polerovirus - P3a - Viral movement - Protein-protein interaction - Cell trafficking.

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C-08

eIF4E1 precision editing provides a solution to the trade-off between the plant development and potyvirus resistance albeit with a low durability

Jean-Luc Gallois (jean-luc.gallois@inrae.fr), Kyoka Kuroiwa, Anaë Laurens, Caroline Lebaron, Marianne Mazier

GAFL, INRAE, Montfavet, France

The development of resistance by loss-of-susceptibility can be undermined by the fact that viruses hijack important components of the plant cell machinery. For example, in Tomato, resistance to the PVY N605 isolate can be achieved by knocking out both *eIF4E1* and *eIF4E2* genes, but is associated with profound effect on the plant development (Gauffier et al. Plant J., 2016). A solution is to design functional alleles encoding host proteins which retain their initial role in the plant physiology but that the virus cannot recruit any longer. Recently, we showed as a proof of concept the conception of an *eIF4E1*resistance allele in cherry tomato obtained by genome editing based on the knowledge resulting from the study of natural functional alleles in crops (Kuroiwa et al., PBJ, 2024). Here, we further assess this allele for its effect on plant development and resistance durability.

We launched a whole-plant phenotyping on a set of tomato genotypes where the endogenous *eIF4E1*gene is edited in the two regions acknowledged to be involved in resistance to potyviruses. We show that the *eIF4E1*-editing in tomatoes do not affect the plant growth and development in a greenhouse setting. We proceeded with the evaluation of the virus resistance durability. We show that most resistant edited *SleIF4E1* suppresses the PVY N605 viral accumulation to a significantly lower level than the wild-type *SleIF4E1* but that this resistance can be broken down substantially whithin nine weeks after inoculation. This is confirmed by the virus obtaining signature mutations of resistance breaking.

Altogether, this work assesses the efficacy of resistance breeding strategy by the base-editing in tomato *SleIF4E1* in an applicational point of view, but calls for further improvement in the resistance allele design and obtention.

Mots clés : Potyvirus - eIF4E - Genome Editing - Tomato.

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C-09

Estimation des nombres de reproduction de base et effectif de la sharka

Norman Daurelle[1, 2] (norman.daurelle@inrae.fr), Anne Quillévéré-Hamard[2], Gaël Thébaud[1], Olivier Cotto[1]

[1] *PHIM, INRAE, Montferrier-sur-Lez (Montpellier), France*
[2] *Epidémiologie et appui à la surveillance, ANSES, Lyon, France*

Les décideurs utilisent fréquemment des indicateurs pour synthétiser les informations relatives à des situations et des processus complexes, facilitant ainsi l'élaboration de stratégies visant à atteindre des objectifs définis. En épidémiologie, le nombre moyen de cas secondaires générés par une infection initiale dans une population sensible, ou nombre de reproduction de base d'une maladie, noté *R*0, mesure le potentiel épidémique d'un agent pathogène. Cet indicateur permet notamment d'estimer la taille maximale et finale d'une épidémie, ainsi que l'intensité des mesures de gestion à mettre en œuvre pour en empêcher le développement. Le *R*0 a rarement été étudié pour des maladies vectorielles des plantes. Ici, nous examinons le *R*0 de la sharka, maladie causée par le plum pox virus et transmise aux *Prunus* (abricotiers, pêchers, pruniers, etc.) par des pucerons. Cette maladie peut causer des pertes économiques importantes, mais son *R*0 n'a jamais été estimé. Dans ce but, nous utilisons des méthodes adaptées aux caractéristiques des différentes données disponibles. La quasi-stationnarité des séries temporelles d'incidence issues de la surveillance permet d'obtenir des estimations de *R*0 à partir de l'incidence attendue à l'équilibre dans un modèle épidémiologique SEIR non spatialisé. Nous montrons que la paramétrisation de ce modèle avec des estimations issues de données spatialisées aboutit à des estimations de *R*0 différentes des premières. Nous utilisons également des simulations de la propagation de la maladie dans un ensemble de vergers pour obtenir des estimations de *R*0 dans différents contextes spatiaux. L'intérêt et les limites de ces approches seront présentés.

Mots clés : Épidémiologie - Modélisation - Nombre de reproduction de base - SEIR - Plum pox virus.

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C-10

Relations entre virulence, charge virale et transmission du potato virus Y (genre *Potyvirus* ; famille Potyviridae)

Lola Chateau[1] (lola.chateau@inrae.fr), Lucie Tamisier[1, 2], Thomas Oudin[1], Roxanne Barosi[1], Marion Szadkowski[1], Grégory Girardot[1], Benoît Moury[1]

[1] *Pathologie végétale, INRAE, Montfavet, France*
[2] *GAFL, INRAE, Montfavet, France*

Le potato virus Y (PVY) est un agent pathogène causant des pertes économiques dans les cultures de solanacées. Il est transmis de manière non persistante et non circulante par des pucerons vecteurs, tels que *Myzus persicae*.
Afin de mieux comprendre l'évolution du PVY, nous avons analysé les relations entre la charge virale présente dans les plantes infectées, l'efficacité de sa transmission par *M. persicae* et sa virulence (ou agressivité), estimée par la réduction de masse fraîche des plantes induite par l'infection virale. Ces variables ont été mesurées pour 16 isolats représentatifs de la diversité du PVY et chez un génotype de piment.
Des différences significatives ont été mises en évidence entre isolats de PVY et cela pour chacune des trois variables mesurées. Ainsi, nous avons mis en évidence une corrélation positive significative, bien que faible, entre la charge virale et la virulence. Nous avons également observé une relation de type quadratique entre virulence et transmission. Ce modèle prédit des relations opposées entre transmission et virulence en fonction de l'efficacité de transmission : relation négative pour les isolats faiblement transmis et positive pour les isolats fortement transmis. Enfin, nous n'avons pas observé de relation entre charge virale et transmission
La faible corrélation entre charge virale et masse des plantes suggère une forte tolérance du génotype de piment testé. Par ailleurs, l'absence de relation entre charge virale et transmission suggère que d'autres facteurs influencent la transmission (comme l'accessibilité du virus ou le comportement du puceron).
Enfin, l'hypothèse du «trade-off,» propose qu'il existe un niveau de virulence optimal permettant de maximiser la transmission d'un parasite. Nous n'avons pas observé cette relation. De plus, cette hypothèse suppose que chacune des variables « transmission » et « virulence » soit corrélée positivement à la charge virale, ce que nous n'observons que partiellement dans notre étude.

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C-11

Does high virus prevalence in wild plants mean beneficial co-evolution? A case study on banana mild mosaic virus

Fanny Leleux (fanny.leleux@doct.uliege.be), Eline Junique, Sébastien Massart

Integrated and Urban Plant Pathology Laboratory, Gembloux AgroBioTech, ULiege, Gembloux, Belgique

Banana mild mosaic virus (BanMMV) infects around 40% of wild plants from the *Musa* genus gathered during collecting missions. Such viral prevalence in a competitive ecosystem between plants raises the question of the co-evolution and putative beneficial interactions between BanMMV and its host plants. The beneficial impact of plant viruses on their host has already been demonstrated for tolerance to abiotic stresses like drought, heat, cold, or salt. To investigate our hypothesis, we analyzed the growth of in-vitro banana plants infected or not by BanMMV in three different abiotic stress conditions: heat and shade stress (based on the host ecological conditions) and drought stress (based on the literature on overlapping defense signaling pathways).
The first bioassay was conducted for 40 days on shade-stressed plants (85% shading net covering the in-vitro tubes). The second bioassay took place for 53 days with plants put under drought stress (simulated by reducing the water availability by adding 0.2M sorbitol in the in-vitro medium) or heat stress (grown at 32°C, 6°C higher than the control group). At the end of the experiments, height, fresh and dry weight as well as the number of shoots and leaves were assessed.
All three stresses had significant impacts on the plants, with a higher height due to the lack of light for shade stress, a higher number of shoots in heat stress, and a lower height and number of leaves in drought stress. Data is still being processed but preliminary results show that BanMMV infection did not cause any significant difference in shade or heat stress. Regarding drought stress, the BanMMV-negative plants were significantly impacted compared to control plants regarding their height and number of leaves, while BanMMV-positive plants were not. These preliminary results will further be investigated through pot experiments and, if confirmed, their molecular mechanisms explored.

Mots clés : Banana - BanMMV - Mutualism - Abiotic stress - Interaction.

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RÉSUMÉS DES COMMUNICATIONS

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K-03

Unveiling the molecular mechanisms and the biological effects associated with infectious agents with viroid-like properties (ribozycirculome) in fungi

Marco Forgia[1] (marco.forgia@ipsp.cnr.it), Cristina Formiglia[1], Yi Guo[1], Stefania Daghino[1], Nadia Serale[6], Emanuela Gobbi[2], Virgilio Balmas[3], Laura Bertini[4], Carla Caruso[4], Marcos de la Peña[5], Francesco Di Serio[6], Beatriz Navarro[6], Massimo Turina[7]

[1] *Institute for Sustainable Plant Protection, National Research Council of Italy, Torino, Italy*
[2] *Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy*
[3] *Department of Agricultural Sciences and NRD - Desertification Research Center, University of Sassari, Sassari, Sardegna, Italy*
[4] *Department of Ecological and Biological Sciences, University of Tuscia, largo dell'Università snc, Viterbo, Italy*
[5] *Instituto de Biología Molecular y Celular de Plantas, Universidad Politécnica de Valencia-CSIC, Valencia, Spain*
[6] *Institute for Sustainable Plant Protection, National Research Council of Italy, Bari, Italy*
[7] *Institute for Sustainable Plant Protection, National Research Council of Italy, Brescia, Italy*

Mycoviruses (viruses infecting fungi) are known since the 60' due to the pathogenic potential on edibles mushrooms, but the research in this field had a rapid increase after the discovery of the virus-induced hypovirulence in the chestnut pathogen *Cryphonectria parasitica*. Following studies on this topic showed that the interest in mycoviruses is not only related to the potential as biocontrol agent against fungal pathogens, but also to the many hidden phenotypes unveiled when focusing on the tripartite interaction between the virus, the fungal host and the environment of origin (or a fungal-interacting organism). Nevertheless, mycoviruses seems to exhibit a remarkable diversity, and studies on the virome of fungal collections revealed several divergent viral taxa never reported before. Recently, a new group of RNA replicators showing circular RNA genomes and containing ribozymes involved a in symmetric rolling cycle replication mechanism were detected and named ribozycirculome. Among these entities, we have been able to characterize a new group of viroid-like entities coding for an RdRp, responsible for the genome replication, that were called ambivirus, and several viroid-like RNAs infecting different fungi. Through a reverse genetic approach, the molecular aspects involved in the replication of the viroid-like entities and the biological effect on the hosts are here described using as a model two ambiviruses isolated from *C. parasitica* and *Fusarium graminearum*, and a viroid-like RNA from Trichoderma spirale.

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C-12

Deciphering the complex ecology of plant and mycoviruses in wild grasses by analyzing the virome associated with individual plants

François Maclot (francois.maclot@uliege.be), Sébastien Massart

Phytopathologie intégrée et urbaine, Université de Liège - Gembloux Agro-Bio Tech, Gembloux, Belgique

Before the domestication of plants, plant viruses were co-evolving with wild plants growing in mixed species communities, thereby potentially resulting in complex interactions (antagonism, commensalism, mutualism). The development of agriculture deeply modified ecosystems, altering the dynamics of virus-plant pathosystems and accelerating the rate of virus evolution and emergence. High-throughput sequencing technologies have now enabled more comprehensive studies of viromes at different scales, from individual plants to entire ecosystems, offering insights into virus ecology within agro-ecological landscapes.
Recent virome studies revealed diversified and largely unknown viral communities in natural ecosystems, with high rates of co-infection and a high abundance of persistent or cryptic viruses. However, many studies focused on plant pools, showing the viral richness but missing important ecological information such as viral prevalence, co-infection and spatial distribution of virus infection. In this context, we conducted a study in the Natural Park “Burdinale-Mehaigne” (Belgium) to explore virus diversity and ecology in individual plants and pooled samples of two grass species, *Lolium perenne* and *Poa trivialis*, from pastures and high biological value grasslands.
Using a virion-associated nucleic acids (VANA) metagenomic approach on 143 individual plants, the study found for both host species a higher virus prevalence (79%) in pastures, dominated by phytoviruses, whereas grasslands showed lower virus prevalence (48%), with a predominance of mycoviruses. Nevertheless, low levels of virus co-infection were observed. For lolium latent virus and yellow dwarf viruses (B/CYDVs), the comparison of sequenced genomes within and between plants suggested a large genetic diversity. Additionally, *Lolium perenne* was identified as a key virus reservoir, hosting up to 25 different virus species compared *Poa trivialis* infected by up to 16 viruses. These findings underscore the significant role of wild plant communities as virus reservoirs, influencing virus ecology across both natural and agricultural landscapes.

Mots clés : Virus ecology - Wild grasses - Virus metagenomics.

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C-13

Mapping cell-type specific localization of plant viruses and effectors in aphid salivary glands with advanced FISH techniques

Laura Di Pietro[1] (laura.di-pietro@umontpellier.fr), Bastien Cayrol[2], Marilyne Uzest[2], Christine Sidobre[3], Stéphane Blanc[2]

[1] *PHIM, Cirad de Baillarguet, Université de Montpellier, Montferrier-sur-Lez, France*
[2] *PHIM, Cirad de Baillarguet, INRAE, Montferrier-sur-Lez, France*
[3] *PHIM, Cirad de Baillarguet, CNRS, Montferrier-sur-Lez, France*

The salivary glands (SG) of aphid vectors are the ultimate barrier to the transmission of circulative plant viruses, inoculated together with the saliva to colonize new host individuals. Structurally, the SG consist of two principal salivary glands (PSG), each connected to an accessory salivary gland (ASG). While it is known that the expression and secretion of the c002 and SHP salivary effectors are restricted to specific cell types within the PSG, very little information is available on whether circulative viruses may also exhibit a cell-type specific localization. The underlying reason of such PSG cell-type restriction is completely unknown and a first step appears to be the mapping of more effectors and viruses. Investigating whether the different cell types within the SG serve distinct functions could provide significant insights not only into the complex mechanisms of production of many distinct types of saliva, but also on how viruses may use some of them very specifically. This study develops advanced hybridization chain reaction (HCR) DNA fluorescent in situ hybridization (FISH) and HCR RNA FISH techniques to precisely co-localize viral DNA or RNA and a variety of effectors. We initially investigated the localization of the nanovirus faba bean necrotic stunt virus (FBNSV) within the PSG cells of *A pisum*. The FBNSV was localized in two large secretory cells (cell type 8) along with other cells expressing the *c002* effector (cell types 3 and 4). We are currently extending the study to additional effectors and viruses, including the capulavirus alfalfa leaf curl virus (ALCV; *Geminiviridae*), and the polerovirus turnip yellow virus (TuYV, *Solemoviridae*). The findings will generally inform on functional complexity of aphid salivary glands and more specifically on how viruses may use this complexity eventually targeting cells producing specific effectors.

Mots clés : Virus - Vector-transmission - Aphid - Plant viruses - HCR RNA/DNA FISH.

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C-14

Canine detection of plant viruses: a proof-of-concept with the plum pox virus

Sylvie Dallot[1] (sylvie.dallot@inrae.fr), Anne Xuéreb[2], Emmanuelle Chiche[3], Gaël Thébaud[1]

[1] *UMR PHIM, INRAE, Montpellier, France*
[2] *UMR CBGP, INRAE, Montferrier-sur-Lez, France*
[3] *DOG NOSE, Montpellier, France*

Over the past two decades, the use of sniffer dogs has expanded rapidly, particularly in the fields of conservation biology and medical diagnosis. Plant health surveillance systems could also benefit from this low-tech, real-time and mobile detection “system” that can be deployed over vast areas and in a variety of environments. The ability of dogs to detect phytopathogenic bacteria and fungi has recently been demonstrated, but their performance in detecting plant viruses is still undocumented. We developed rigorous training and evaluation designs to assess the performance of two dogs in detecting peach plants infected by the plum pox virus (PPV; *Potyvirus plumpoxi*), responsible for a serious viral disease of *Prunus* trees that is currently managed using symptom inspections. The dogs were trained to discriminate symptomatic PPV-infected peach plants from certified virus-free plants and from plants infected by PDV, PNRSV and ACLSV. Based on preliminary data collected during training, we used simulations to identify an experimental design allowing good statistical power (precision and accuracy) for performance estimates (detection sensitivity and specificity) while retaining experimental feasibility. Twenty runs of 18 plants each, among which the number of PPV-infected plants was randomly assigned, were presented to the dogs in a double-blind fashion to prevent behavioral bias. Detection performance reached 85% sensitivity and 99% specificity for one dog. In a second step, canine detection performance was evaluated using devices that captured volatile organic compound (VOC) during 3-4 days on the same plants in full vegetation and at bud break. For both dogs, detection performance using VOC-capture devices was excellent for plants in vegetation (86.5% sensitivity and 92% specificity) but was very poor for plants at bud break. The ability of dogs to detect weak VOC signals (winter dormancy, latent infections) needs further evaluation.

Mots clés : Sniffer dog - Volatile organic compounds - Phytovirus.

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C-15

Exploring a new territory for grapevine viruses: first outputs from winemaker interviews and virome survey in Belgium

Lavena Van Cranenbroeck[1] (lavena.vancranenbroeck@doct.uliege.be), Fauve Maertens[2], Maaike Heyneman[2], Nuria Fontdevila Pareta[1], Matthias Praet[2], Tanguy Campillo[1], Loris Caillat-Miousse[1], François Maclot[1], Serkan Önder[1, 3], Kris De Jonghe[2], Sébastien Massart[1]

[1] *Laboratoire de Phytopathologie Intégrée et Urbaine, Gembloux Agro-Bio Tech Université de Liège, Gembloux, Belgique*
[2] *Plant Sciences Unit, Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Merelbeke, Belgique*
[3] *Department of Plant Protection, Faculty of Agriculture Eskişehir Osmangazi University, Eskişehir, Türkiye*

Belgian viticulture, driven by climate change and growing consumer interest, has expanded from 72 hectares in 2006 to 891 hectares in 2023. Despite lacking a long-established winemaking tradition, Belgium is creating new agroecosystems where winemakers explore diverse grape varieties and cultural techniques.
However, this expansion in new territories brings growing threats from viral and viroid pathogens. Key questions arise: Which viruses and viroids could potentially affect Belgian vineyards? Are they already present in our territory? How did they arrive? What is the readiness for outbreak management?
To address these concerns, we conducted a comprehensive study involving a questionnaire and interviews with winegrowers. The responses from 88 growers described the typology of grapevine cultivation in Belgium related to the cultural practices, the origin of planting material, the diversity of cultivars, the perception of viral and phytoplasma threat… This survey was complemented by an extensive virome survey: more than 7,000 plants (either symptomatic or not) from 50 grape varieties were sampled in 86 vineyards across Belgium. For asymptomatic samples, double-stranded RNA (dsRNA) extraction was performed, while total RNA extraction was conducted on symptomatic leaves. The RNA extracts were then sequenced and relevant virus detection confirmed by RT-PCR.
Analysis of symptomatic samples collected over the past two years identified 6 viruses and 4 viroids for the first time in Belgium, including Grapevine fanleaf virus (GFLV), all confirmed independently by RT-PCR. Asymptomatic samples analysis is ongoing.
Further investigations will explore potential links between the observed virome and the typology of vineyards, including plant origin and cultivar. Beyond scientific results on viruses, the interactions with growers during the virome survey is also raising awareness about the viral (and phytoplasma) risks. This research enhances our understanding of phytosanitary risks in Belgian viticulture and contributes to developing effective management strategies to safeguard this emerging industry.

Mots clés : HTS - Belgian viticulture - Vitis vinifera - Phytosanitary risk - Growers survey.

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K-04

Multiscale modelling of arthropod-borne virus transmission dynamics

Pauline Ezanno (pauline.ezanno@inrae.fr), Léa Loisel, Gaël Beaunee, Hélène Cecilia

Oniris, INRAE, BIOEPAR, Nantes, France

Many infectious diseases originate from animals. Among those, 60% end up affecting humans, seriously threatening food supplies, the global economy, and veterinary public health. Vector-borne diseases (VBD) are of particular interest, as they are expanding their geographical distributions due to global changes, threatening new populations. In epidemiology, you should first get to know your enemy to fight it effectively. For VBD, understanding the impact of abiotic (e.g., temperature, humidity) and biotic (e.g., species) factors on host and vector abundance, geographical distribution, and contacts is of utmost importance as it can profoundly shape virus spread. These are commonly studied using epidemiological modelling, which is extremely useful for identifying appropriate management strategies. However, models have so far too often neglected infection processes happening at the micro-scale, such as viral load dynamics and immune responses. Indeed, relationships among within- and between-host processes are still poorly understood. Therefore, there is a need to reconcile and integrate scales, built on interdisciplinary research efforts, to better understand, anticipate, and control diseases. Such collaborations are key in deciphering when and how to combine the intra-individual and the population scales in mechanistic models. In this presentation, using major zoonotic VBD as a common thread, we will illustrate how much host and vector responses to viral infections are heterogeneous, and how such heterogeneity at individual scale could shape epidemics. This will lead us to reconsider the ability of VBD epidemiological models, most of which consider large-scale processes only, to support surveillance and decision making. We will discuss the possible need for a multiscale modelling framework to assess the reciprocal interactions among processes occurring at individual and population scales. This would require greater collaborations between virologists / immunologists and epi-modelers / bio-mathematicians. We will conclude on the potential added value of data centralization, documentation and sharing.

Keywords: Multiscale - Viral dynamics - Mechanistic model - Vector-borne diseases.

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C-16

The spicy dissemination of cucumber mosaic virus in Espelette

Loup Rimbaud[1] (loup.rimbaud@inrae.fr), Karine Berthier[1], Lola Chateau[1], Alice Conilh[1], Cécile Desbiez[1], Romane Ducasse[1], Paul Dumeaux[2], Jonathan Gaudin[1], Grégory Girardot[1], Lucas Gonzalez[1], Judith Hirsch[1], Elise Lepage[1, 3, 4], Benoît Moury[1], Elodie Nguyen[2], Sylvain Piry[1, 5], Alexandra Schoeny[1], Marion Szadkowski[1], Claire Troulet[1], Eric Verdin[1], Catherine Wipf-Scheibel[1]

[1] *Pathologie Végétale, INRAE, Montfavet, France*
[2] *Syndicat du Piment d'Espelette AOP, Espelette, France*
[3] *BioSP, INRAE, Avignon, France*
[4] *AgroParisTech, Palaiseau, France*
[5] *UMR CBGP, INRAE, Montferrier-sur-Lez, France*

Espelette pepper farmers are currently facing devastating and recurrent viral epidemics due to a recent emergence of *Cucumber mosaic virus* (CMV). A collaboration between the "Syndicat du Piment d'Espelette AOP" (which federates Espelette pepper farmers) and the Virology team of the Plant Pathology unit of INRAE (Avignon) has emerged to investigate these epidemics and propose control strategies. To identify relevant and promising strategies, it is first necessary to acquire data on the virus epidemiology in the local context and understand what drives CMV epidemics in the Espelette area. In particular, it is currently unclear how CMV re-emerges every year on pepper crops despite the absence of pepper during winter. It is also intriguing to observe such severe epidemics in this area, where the high level of landscape fragmentation and plant biodiversity is generally predicted to mitigate disease spread.
In this study, we explored the possibility of year-to-year seed transmission from infected fruits, of the presence of alternative hosts (serving as viral reservoir and thus inoculum sources) around pepper fields as well as the role of aphid vectors (mediating plant-to-plant transmissions). For this, we first performed large-scale surveys to collect samples (seeds, plants, aphids) in the Espelette area and monitor CMV incidence in different elements of the agricultural landscape (pepper crops, surrounding crops, wild plants). Second, we ran greenhouse experiments in controlled conditions to assess the efficiency of seed- and aphid-mediated transmission.
This work enables the evaluation of the respective roles of the different CMV dissemination pathways and helps decipher the key drivers of CMV epidemics in the Espelette area. These results will guide future research efforts and help prioritize management options for further investigation.

Mots clés : Cucumovirus - Espelette pepper - Viral transmission - Wild reservoir - Capsicum annuum.

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C-17

A virus-induced gene silencing (VIGS) approach to study plant-geminivirus-insect vector interactions and virus transmission

Fani Gousi[1] (fgousi@gmail.com), Cica Urbino[1], Martine Granier[1], Nathalie Laboureau[1], Jyoti Singh[2], Vipin Hallan[2], Michel Peterschmitt[1], Mikhail M. Pooggin[1]

[1] *PHIM Plant Health Institute, Univ. Montpellier, CIRAD, INRAE, IRD, Montpellier, France*
[2] *CSIR-Institute of Himalayan Bioresource Technology, Palampur, Himachal Pradesh, India*

Geminiviruses (family Geminiviridae) are plant viruses with circular ssDNA genomes encapsidated in geminate virions and transmitted by insect vectors in a persistent circulative manner, causing severe crop diseases globally. Gemiviruses of the genus Begomovirus (begomoviruses) are often associated with circular ssDNA betasatellites encoding a protein that suppresses plant RNA interference (RNAi)-based defenses and innate immunity. Geminiviruses replicate in plant cells through rolling circle replication (RCR) and recombination-dependent replication (RDR), generating circular ssDNA for encapsidation and linear concatemeric dsDNA, respectively. RDR products may serve for resurrection of cirlular ssDNA via RCR and/or for evasion of the antiviral RNAi generating virus-derived small RNAs. Recent studies showed that Tomato yellow leaf curl virus (TYLCV) can replicate in the salivary glands of its insect vector Bemisia tabaci (whitefly), which ensures persistent infection and efficient transmission to new plants. This discovery raises questions about the underlying replication mechanisms and how the whitefly antiviral RNAi and innate immunity respond. To validate the function of host plant and insect vector genes implicated in begomovirus replication and transmission, we applied a virus-induced gene silencing (VIGS) approach. We designed a disarmed betasatellite vector lacking an RNAi suppressor, for carrying inserts of candidate plant and insect genes. Initial tests confirmed efficient replication of empty VIGS constructs in host plants and successful transmission by whiteflies. Using VIGS to knock down expression of the plant PCNA gene essential for DNA replication led to severe developmental abnormalities, unlike controls. Current experiments involve silencing of the whitefly PCNA gene implicated in begomoviral DNA replication and two other whitefly genes implicated in begomovirus circulation and transmission. The results of these experiments will be presented and discussed. As an ultimate goal, we aim to develop new strategies for the control of viral disease spread at the transmission step without affecting insect viability.

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C-18

Genomic insights into the global emergence of the phytopathogenic Maize yellow mosaic virus

Damien Richard[1] (damien.richard@ird.fr), Palanga Essowè[2], Nils Poulicard[1], Eugénie Hébrard[1]

[1] *PHIM, Université Montpellier, IRD, INRAE, Cirad, Institut Agro, Montpellier, France*
[2] *ITRA, Centre de Recherche Agronomique de la Savane Sèche (ITRA-CRASS), Kara, Togo*

Maize yellow mosaic virus (MaYMV) is an emerging Polerovirus that was first described on Maïze in China in 2016. It has been subsequently described on other crops in three continents. Only limited genetic resources are available for this virus and knowledge about its ecology and evolutionary history is still scarce. In order to better characterize the past evolution of MaYMV and its genetic structure, we assembled a large dataset of 400 complete genomes sampled globally. We leveraged off target reads of plant-derived RNA metagenomes, showcasing the value of these datasets for RNA virus genomics. Using a suite of phylogenetics tools, we highlighted a strong geographic structure in three clades corresponding to Africa, America and Asia, linked to the virus' ecology. We inferred the molecular clock of the virus and dated several key evolutionary events linked to its emergence. We report both known and undescribed hosts including multiple cultivated and wild Poaceae species and also expand the known geographic distribution of the virus. Our results also point to a lack of host adaptation, suggesting all known lineages of this viral pathogen display a broad host range including several Poaceae species.

Mots clés : Emergence - Molecular dating - Poaceae - Datamining - Polerovirus.

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C-19

Integrative Proteomics for Predicting Biological Properties of Emerging Plant Viruses: A Genomics-Driven Machine Learning Framework

Nikolay Simankov[1, 2] (nikolay.simankov@doct.uliege.be), Rachid Tahzima[1, 3], Hélène Soyeurt[2], Sébastien Massart[1]

[1] *Laboratory of Plant Pathology, Teaching and Research Centre, Gembloux Agro-BioTech, ULiège, Gembloux, Belgium*
[2] *Statistics, Informatics and Applied Modeling (SIMa), Teaching and Research Centre, Gembloux Agro-BioTech, ULiège, Gembloux, Belgium*
[3] *Flanders Research Institute for Agriculture, Plant Sciences, Fisheries and Food, Merelbeke, Belgium*

The wide application of high throughput sequencing technologies has led to the discovery of hundreds of new plant viruses. Their biological characterization, mandatory to evaluate the risk they can cause, is now a significant bottleneck. To limit this bottleneck and to improve the efficiency of biological characterization, our research aims to use machine learning (ML) approaches to proteomics annotations of newly discovered viruses to predict their biological properties. So, we exploited a database of biological (transmission pathways, host range) and conserved functional and structural proteomic motifs (Pfam, GO, CATH, SSF) for a representative set well-characterized plant virus species. Applying a combination of supervised ML methods and appropriate data wrangling approaches allowed us to build predictive models with Cohen's Kappa scores ranging between 89.8% and 100% for predicting vector-borne transmission. As biological datasets are sensitive to missing (biological) information, our study integrated an innovative methodology to identify the missing information. This allowed us to predict, vectors such as planthoppers, aphids, and whiteflies with Cohen's Kappa scores of 99.8%, 92,5%, and 92,3%, respectively.
To further refine our predictive framework, we augmented the database with physicochemical properties (n=618) and short amino acid k-mer frequencies (n=8440) of all encoded viral proteins in the RefSeq database. Currently, we are evaluating how these detailed molecular features could enhance the model's prediction accuracy, including for the plant host range. Beyond the applied predictions for risk analyses, our ML-based approach could also provide novel insights and research hypotheses to accelerate our understanding of the relationship between plant virus proteomes and their host specificity.

Mots clés : Plant Viruses - Machine Learning - Host Specificity - Proteomics - High Throughput.

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C-20

Get to know each other: Considering the genetic diversity of grapevine fanleaf virus at a local scale prior to a cross-protection trial in the field

Pierre Mustin[1] (pierre.mustin@inrae.fr), Jean-Michel Hily[2], Julie Kubina[1, 3], Shahinez Garcia[1, 3], Véronique Komar[1], Claude Gertz[1], Wassim Rhalloussi[1, 2], Gérard Demangeat[1], Olivier Lemaire[1], Anne Sicard[1], Emmanuelle Vigne[1]

[1] *Santé de la Vigne et Qualité du Vin, INRAE, Colmar, France*
[2] *IFV, Le Grau-du-Roi, France*
[3] *Institut de Biologie Moléculaire des Plantes, CNRS, Université de Strasbourg, Strasbourg, France*

Cross-protection is usually defined as a phenomenon in which a primary infection of a host plant by a "cross-protecting" virus prevents its subsequent infection by a genetically related "challenge" virus. Previous research on different viral pathosystems points towards the importance of phylogeographic structuration of virus populations as a constraining factor for the success of cross-protection in the field. As grapevine fanleaf virus (GFLV) populations have recently been shown to be very genetically diverse and geographically structured, we aim at taking into account the local genetic diversity of potential cross-protecting and challenger GFLV isolates to experiment cross-protection against this pathogen in the field. Therefore, among 5595 plants grown in a highly infected plot in Burgundy, we looked for GFLV-infected vines, displaying no or low symptoms and satisfying yields (i.e. candidate vines), with no significant differences to GFLV-free vines. Furthermore, within this same plot, we identified an area of 200 plants that is 93% GFLV infected, exhibiting severe symptoms and thereby constituting a promising future trial area. Using High Throughput Sequencing techniques, 59 GFLV consensus sequences found in 22 candidate vines were compared to potential challengers that are 158 GFLV sequences infecting the vines grown in the future trial area. It revealed a complex genetic diversity where about a quarter of potential cross-protecting GFLV sequences belonged to the same phylogenetic clades as potential challenger GFLV sequences (93.9% to 99.8% of sequence identity) while others were unique to both groups (around 88.6% of sequence identity). Finally, the estimation of the genetic relatedness between potential cross-protecting and challenger GFLV isolates enabled us to set up a pioneer cross-protection trial, wherein GFLV primary infected vines will be phenotypically and genetically monitored to assess their degree of protection under their original field conditions.

Mots clés : Cross-protection - Grapevine fanleaf virus - High Throughput Sequencing - Field.

C-21

Phenotypic characterization of the resistance observed in *Oryza glaberrima* accession against RSNV

Emma-Louise Jaffré[1] (emmalouise.jaffre@ird.fr), Camille Blasco[1], Adeline Pinault[1], Lucie Hugues-Perez[1], Agnès Pinel-Galzi[1], Cécile Desbiez[2], Eugénie Hébrard[1], Laurence Albar[1]

[1] *PHIM, IRD, Montpellier, France*
[2] *Pathologie végétale, INRAe, Avignon, France*

The rice crinkling disease has been observed in West Africa and South America, and has the potential to cause significant yield losses, reaching up to 80% in localized epidemics (Bagayoko et al. 2021). The causal agent is *Benyvirus oryzae*, also known as *Rice stripe necrosis virus* (RSNV; *Benyviridae, Benyvirus*), a bipartite virus composed of two positive single-stranded RNA segments. RSNV is transmitted to rice (*Oryza* spp.) by *Polymyxa graminis* f. sp. c*olombiana* (Pgcol), a soil protist that belongs to the *Plasmodiophoridae*family. *Pg. col* produces sporosores, that enable it to survive for up to a decade in soil. The tripartite pathosystem remains poorly understood, and no sustainable control measures have yet been developed. Nevertheless, a resistance source against this disease was observed in *O. glaberrima* accession MG12 (Gutierrez et al. 2010) under field conditions. It may be associated with a major resistance gene that has not yet been identified and introgressed into high-yielding agronomic varieties. One of our objectives is to characterize this resistance phenotypically under controlled conditions. The viral multiplication of RSNV is being compared in leaves and roots of MG12 and susceptible rice varieties, based on serological and molecular methods. In parallel, the *Pg. col* colonization of roots is being compared by microscopy in order to determine whether resistance is targeted against the virus or the vector. The laboratory multiplication of *Pg. col* is a lengthy and intricate process, therefore, an infectious clone of RSNV is being developed to facilitate resistance phenotyping. The clones carrying the two RNAs have been obtained by homologous recombination in yeast and their infectivity is now being assessed through different methods on rice, *N. benthamiana* and *Chenopodium* spp.

Mots clés : Phenotyping - Tripartite interaction - Resistance sources - Infectious clone - Viral multiplication.

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C-22

Tissue-Specific Variations in the Frequency Distribution of a Multipartite Virus Genomic Components

Prune Lacôte-Popovic[1] (prune.lacote_popovic@outlook.com), Andy Brousse[2], Christine Sidobre[2], Stéphane Blanc[1], Yannis Michalakis[2]

[1] *PHIM, INRAE, CIRAD, Montpellier, France*
[2] *PHIM, MiVEGEC, IRD, CNRS, CIRAD, Montpellier, France*

The genome of multipartite viruses is made of a set of complementary nucleic acid segments, each encapsidated separately in its own virus particle. Those distinct particle types, each type containing on type of segment necessary for either viral replication, countering plant defenses, cell-to-cell or long-distance movement, and vector-transmission, do not accumulate equally upon systemic infection. Instead, the relative frequency of each segment reaches a specific equilibrium, yielding a pattern of segment frequency named “genome formula”. In Nanoviruses, the distribution of segment frequencies converges in a highly reproducible manner across leaf stages to the genomic formula within the same host species. However, it is specific to the host species tested and thus changes when the virus switches hosts. This suggests that differences in the environment of the virus, e.i. the host plant genotype and perhaps also plant physiological parameters may influence the genome formula. In order to further characterize the factors impacting the genome formula of the faba bean necrotic stunt virus (FBNSV), and particularly to assess the impact of distinct organs or tissues, a comparative study was conducted in leaves and roots of large sets of inoculated plants. The FBNSV genome formula was estimated in samples from leaves and secondary roots by quantitative PCR (qPCR). The experiment was repeated using three viral genotypes as well as three distinct host species.
It was demonstrated that for the three FBNSV isolates, JKI-2000, AZ10;12b and AZ15, and across the three host plants, *Vicia faba*, *Lens culinaris* and *Vicia sativa*, the genome formula in roots differ significantly from that in leaves. Notably, the segment U1 accumulated at a much greater frequency in roots as compared to shoots, in all conditions tested. Thus, the genome formula of FBSNV varies with viral genotype, host species and host tissue.

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C-23

Cucumber mosaic virus degrades pepper fruit production, marketability and organoleptic quality, with isolate-specific effects

Elise Lepage[1, 2, 3] (elise.lepage@inrae.fr), Marion Szadkowski[1], Grégory Girardot[1], Michel Pascal[1], Paul Dumeaux[4], Julien Papaïx[2], Benoît Moury[1], Loup Rimbaud[1]

[1] *Pathologie végétale, INRAE, Montfavet, France*
[2] *BioSP, INRAE, Avignon, France*
[3] *AgroParisTech, Palaiseau, France*
[4] *Syndicat du Piment d'Espelette AOP, Espelette, France*

Over the past decade, recurrent epidemics of cucumber mosaic virus (CMV) have damaged Espelette pepper crops, a product from the French Basque Country with a Protected Designation of Origin (PDO). Of the three main CMV phylogenetic groups, isolates from subgroup IB seem to be responsible for these epidemics, while group II is only sporadically present and subgroup IA is completely absent, although common elsewhere in France.
This study quantifies the impact of three CMV isolates belonging to (sub)groups IA, IB, and II on the production and quality of Espelette pepper (*Capsicum annuum* cultivar "Gorria"). The CMV-susceptible inbred line "Yolo Wonder" was also included as a reference.
First, we monitored fruit production dynamics (number and weight) and found that infection by the IA isolate significantly reduced total yield in both cultivars, while infections by the IB and II isolates had milder or non-significant effects.
Second, we assessed the marketability of Gorria fruits under the PDO label by monitoring defects in size, shape and color. Infected plants produced significantly shorter, more misshapen and discolored fruits, especially with infection by isolate IA.
Finally, we evaluated the organoleptic properties of infected Gorria fruits thanks to a jury trained in PDO quality standards. Peppers infected with the IA isolate were significantly more likely to be rejected compared to healthy controls.
Our results underline that CMV infection degrades both pepper yield and quality, with severity depending on pepper cultivar and CMV isolate. In Espelette, managing current epidemics and preventing introduction of IA isolates is thus crucial.

Mots clés : Cucumovirus - Crop losses - Pepper - CMV.

C-24

Synergistic interactions between the beet mosaic potyvirus and the beet yellows closterovirus decrease transmission of the closterovirus

Souheyla Khechmar (Souheyla.khechmar@inrae.fr), Quentin Chesnais, Véronique Brault, Martin Drucker

Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement, Colmar, France

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 alone 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) showedthat co-infection did not relieve phloem restriction of BYV. Also BtMV tissue distribution was unchanged in co-infected leaves compared to mono-infected ones. BtMV accumulated in periplasmic aggregates in mono-infected and co-infected cells, while BYV formed 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 BYV/BtMV interactions could restrict BYV access to the sieve tubes and reduce its accessibility for aphids and present a model of how co-infection could alter BYV intracellular movement and/or phloem loading and reduce BYV transmission.

Mots clés : Aphid transmission - Co-infection - Sugar beet viruses - Virus localisation - Aphid behavior.

RÉSUMÉS DES COMMUNICATIONS

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Mosquito salivary transmission enhancerS

Julien Pompon (julien.pompon@ird.fr)

MIVEGEC, IRD, Montpellier France

Mosquito-borne flaviviruses infect half a billion people yearly, causing 100 000 deaths and considerable economic losses. Upon mosquito bite, flaviviruses are delivered in saliva and first infect skin cells before spreading systematically, resulting in successful transmission. Multiple studies showed that salivary components enhance skin infection. Here, I will present our journey toward the discovery of the transmission-enhancing function of non-coding flaviviral RNA (i.e., subgenomic flaviviral RNA, sfRNA) and lipids in saliva. First, we showed how sfRNA increases infection rate in saliva by inhibiting the Toll pathway innate immune response in mosquito salivary glands. Second, we elucidated how sfRNA is secreted in mosquito salivary extracellular vesicles by interacting with syntenin, a protein involved in extracellular vesicle biogenesis. Third, we determined that secretion of sfRNA in mosquito saliva is conserved across multiple flavivirus-mosquito species systems and showed how sfRNA increases skin infection by inhibiting the interferon innate immune system. Finally, we discovered how lipids in mosquito salivary extracellular vesicles enhance skin infection and transmission for multiple flaviviruses. Altogether, we characterized the multi-prong function of sfRNA-containing extracellular vesicles in flavivirus transmission.

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C-25

Viral manipulation of lipid droplets: insights from turnip mosaic virus infection in plants

Marguerite Batsale[1] (marguerite.batsale@inrae.fr), Nathalie Arvy[1], Léna Jambou[2], Charlotte Quinteau[1], Luc Sofer[1], Vincent Simon[1], Nathan Doner[3], Robert Mullen[3], Denis Coulon[2], Claire Bréhélin[2], Sylvie German-Retana[1]

[1] *UMR 1332 Biologie du Fruit et Pathologie (BFP), équipe Virologie, INRAE, Université de Bordeaux, Villenave-d'Ornon, France*
[2] *UMR 5200 Laboratoire de Biogenèse Membranaire (LBM), CNRS, Université de Bordeaux, Villenave-d'Ornon, France*
[3] *Department of Molecular and Cellular Biology, University of Guelph, Guelph, Canada*

Over the past decade, there has been an increase in evidence that human positive single stranded RNA (+ssRNA) viruses extensively manipulate the host's lipid metabolism and use lipid droplets (LDs) to enhance energy production and promote viral replication and assembly [1]. LDs are dynamic organelles with a core of neutral lipids surrounded by a phospholipid monolayer associated with enzymes and structural proteins. More than simple storage units, these organelles are key regulators of cellular homeostasis, playing significant roles in lipid metabolism, energy storage, and signal transduction [2]. Recent studies in plants have highlighted the potential role of LDs in plant responses to infections caused by both plant and fungal viruses [3-4]. However, the mechanisms involving LDs in plant-virus interactions remain unexplored.
In this study, we present the first comprehensive analysis of LD accumulation and their recruitment to viral replication compartments (VRCs) during infection by turnip mosaic virus (TuMV, a member of the *Potyvirus* genus) in *Arabidopsis thaliana* and *Nicotiana benthamiana*, using confocal and electron microscopy. In agreement with these results, our mRNA translatome analysis of TuMV-infected and mock-infected *A. thaliana* leaves revealed increased translation of LD structural proteins, LIPID DROPLET ASSOCIATED PROTEINs (LDAPs), a family of proteins crucial for LD biosynthesis and stability. Western blot analyses further confirmed the accumulation of LDAP1 in response to TuMV infection. Moreover, TuMV propagation was significantly impaired in Arabidopsis *ldap1* knock-out mutants and enhanced in LDAP1 overexpressing plants. Our preliminary data suggest that LDAP1 may play roles in both viral movement and replication and therefore represent a key factor in LD recruitment during viral infection. Collectively, our results point to a pro-viral function of LDs in TuMV-infected plants.

Mots clés : Potyvirus - Lipid droplets - VRCs - TuMV - LDAP.

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C-26

Characterization of host quality of non-crop Poaceae species for wheat dwarf virus and *Psammotettix alienus*

Thomas Armand (thomas.armand@inrae.fr), Marlène Souquet, Matthias Rapp, Emmanuel Jacquot

UMR PHIM, INRAe, Montpellier, France

Harvest represents a challenge for the persistence of insect-borne viral diseases in agroecosystems. To overcome this challenge, some viruses infect non-crop plants (i.e. spillover) as reservoirs for future introduction of viruses to newly sown fields (i.e. spillback). The wheat dwarf disease (WDD), one of the most important viral diseases on cereals, is caused by the wheat dwarf virus (WDV) and is transmitted by the leafhopper *Psammotettix alienus*. While the epidemic process of this disease has been studied in crop areas, very few data is available on factors influencing spillover/spillback events. To better understand contribution of non-crop species in the epidemiology of WDD, plant-virus (i.e. infection rate) and plant-vector (i.e. survival and fecundity) interactions were monitored on 20 non-crop Poaceae (NCP) species. Results showed that i) host range of WDV is wider than expected and ii) NPC species can be clustered according to host quality for WDV and/or *P. alienus*. *Bromus hordeaceus* and *Phalaris arundinaceae* (two species with contrasted host quality for WDV and *P. alienus*) were included in multi-host arena experiments to assess the impact of a heterogeneous plant environment on leafhopper preferences and WDV infection. Collected data showed that *P. alienus* prefers *B. hordeaceus* (a poor efficient host for WDV) for oviposition. This could lead to a dilution of viruliferous vectors in environments containing *B. hordeaceus* plants as hosts for WDV. Altogether, results of this work indicate that host quality and the composition of plant populations are important for WDD maintenance in non-cultivated areas.

Mots clés : Epidemiology - Intercropping - Geminiviridae - Persistently transmitted plant virus - Virus ecology.

C-27

Transcriptome and small RNAome profiling reveals the molecular mechanisms underlying plant-nanovirus-aphid vector interactions

Valentin Guyot[1] (valentin.guyot@inrae.fr), Victor Golyaev[1], Natalia Sukhikh[1], Auguste Rajot[1], Alexis Bourdais[2], Fabrice Legeai[2], Stéphane Blanc[1], Mikhail M. Pooggin[1]

[1] *PHIM Plant Health Institute, INRAE, CIRAD, IRD, Institute Agro, University of Montpellier, Montpellier, France*
[2] *IGEPP, INRAE, Institute Agro, University of Rennes, Le Rheu, France*

Faba bean necrotic stunt virus (FBNSV) and Faba bean necrotic yellow virus (FBNYV) are distinct nanoviruses (genus *Nanovirus*, family *Nanoviridae*) whose genome is composed of eight ssDNA components each encoding a single protein. They infect plants of the Fabaceae family and are transmitted by aphid vectors in a circulative non-propagative manner. In order to understand the mechanisms underlying plant-nanovirus-aphid vector interactions, we performed Illumina sequencing profiling of the transcriptome and small RNAome of nanovirus (FBNSV or FBNYV)-infected vs mock-inoculated *Vicia faba* and *Medicago truncatula* plants and *Acyrthosiphon pisum* aphids fed on the respective plants. Bioinformatics analysis of the sequencing data allowed us (i) to identify differentially expressed genes co-regulated by both viruses in plants and aphids, (ii) to map the transcription units of viral mRNAs and compare their relative transcription rates and (iii) to investigate the biogenesis and function of virus-derived 21, 22 and 24 nt siRNAs produced by the antiviral RNA interference machinery in both plants. We found no evidence of viral DNA transcription in aphids but detected low abundance viral siRNAs ingested by the aphids with the phloem sap of virus-infected plants. We also analysed survival rates of the viruliferous vs non-viruliferous aphids and found that both nanoviruses reduced the aphid survival rate. Collectively, our comparative analysis of two distinct nanoviruses reveals both commonalities and differences in their interactions with two distinct plant hosts and one aphid vector.

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C-28

OptiCQua: Optimizing the performance of sanitary diagnosis of *Vitis* spp. submitted to certification and quarantine schemes

Dalia Djaboub[1, 2] (dalia.djaboub@anses.fr), Jean-Philippe Renvoisé[1], Jean-Emmanuel Gerbault[1], Olivier Lemaire[2, 3], Jean-Michel Hily[3, 4], Lorène Belval[1]

[1] *Laboratoire de la Santé des Végétaux, Unité de Quarantaine, ANSES, Lempdes, France*
[2] *UMR 1131 Santé de la Vigne et Qualité du Vin, INRAE-Université de Strasbourg, Colmar, France*
[3] *Laboratoire Partenarial Associé Vitivirobiome, Colmar, France*
[4] *Institut Français de la Vigne et du Vin, Le Grau-du-Roi, France*

France and European Union set-up certification and quarantine measures of *Vitis* spp. to protect the sanitary quality of their vineyards. My doctoral project primarily aims to validate a High Throughput Sequencing (HTS) test for routine sanitary diagnosis of grapevine plants needing to meet health regulatory requirements. To date, the implemented diagnosis schemes rely on methods requiring target's prior knowledge, such as biological indexing, molecular and serological tests. Given that a HTS test could identify the entire plant virome, its implementation would ultimately enable replacing some target-specific tests, plus detecting unknown or newly described viruses and viroids.
I started the project with a protocol optimization, sampling different plant parts and testing different isolation kits. RNA yield and quality results led us to opt for a Qiagen RNeasy Plant mini kit adapted protocol using wood, roots or leaves. After ribo-depletion and library preparation, cDNAs are sequenced using Illumina NovaSeq-6000/X-Plus, generating 150bp paired-end reads. Resulting sequences were analyzed using two bioinformatic pipelines.
In order to validate the optimized test, my work aims to establish its performance criteria, including inclusivity and selectivity, and to compare them to the target-specific tests. I selected a collection of 20 plants infected with regulated RNA and DNA viruses and analyzed the samples using conventional, real time (RT)PCR, or ELISA routine quarantine tests. A biological indexing assay has also been set-up using six plants of the collection, targeting certification viruses. I then sequenced all samples to establish and compare the tests inclusivity. Moreover, I sampled wood, roots and leaves from 14 plants of the collection, and performed their sequencing in order to define the test selectivity and to study the viruses distribution during plant cultivation. These results will allow establishing the analytical sensitivity, repeatability, reproducibility and robustness of the HTS test, to validate it for certification and quarantine use.

Mots clés : Vitis spp. - High throughput sequencing - Certification - Quarantine - Virus.

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K-06

Plant-virus co-evolution during adaptation to the transmission mode

Israel Pagán (jesusisrael.pagan@upm.es)

Centro de Biotecnología y Genómica de Plantas UPM-INIA/CSIC, Campus UPM Montegancedo, Madrid, Spain

Transmission efficiency is a key trait for every pathogen as it ultimately determines fitness. Plant viruses are not exceptions and evolved various ways to achieve transmission. The best characterized is plant-to-plant horizontal transmission, which mainly occur through insect vectors. However, more than 25% of plant viruses are vertically transmitted from-parent-to-offspring through seeds, and for some is the only way to infect new hosts. Most theoretical works predicting how vertical transmission evolves agree in that maximum rates require lower virulence and increased plant fitness. This is because higher number of plant seeds enhances the chances for the virus to be vertically transmitted. Thus, optimizing seed transmission also benefits the host plant. Remarkably there is little support for this prediction, and even less is known on the mechanisms that may mediate the evolution of seed transmission. We have addressed this question by performing serial passages of strict cucumber mosaic virus (CMV) seed transmission in *Arabidopsis thaliana*. After serial passages, CMV seed transmission was increased by one order of magnitude, which was associated to lower virulence and higher virus multiplication in reproductive organs. Hence, adaptation to seed transmission appears to be associated with increased tolerance, rather than resistance, to CMV. In agreement, these changes were explained by modifications in both the virus and the plant. On one hand, CMV accumulated mutations in the 3´untranslated region of RNA3 that altered secondary structures relevant for virus replication and protein translation. On the other hand, *A. thaliana* plants showed differential DNA methylation patterns that mainly affected to genes involved in plant growth, reproduction and defense against pathogens. Notably, hypermethylated (lower expression) regions were more frequently affecting defense genes, whereas hypomethylated (enhanced expression) regions generally occur in growth/reproduction genes. Overall, this work provides new insights on how the mode of transmission determined plant-virus co-evolution.

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C-29

Genetic architecture of *Tobacco mosaic virus* tolerance in *Arabidopsis thaliana*

Julien Hennequart[1] (julien.hennequart@inrae.fr), Mélodie Caballero[1], Manfred Heinlein[2], Todd Blevins[2], Claudia Rouveyrol[1], Adam Rochepeau[1], Pierre Petriacq[1], Valérie Schurdi-Levraud[1]

[1] *UMR 1332 Biologie du Fruit et Pathologie, INRAE, Villenave-d'Ornon, France*
[2] *UPR 2357 Institut de Biologie Moléculaire des Plantes, CNRS, Strasbourg, France*

Resistance to viruses is often effective only in the short term as the high rate of evolution of viruses allows them to bypass resistance mechanisms. Conversely, tolerance (i.e., the ability of plants to accumulate pathogens in their tissues without causing disease symptoms or loss of fitness) may be more durable as the viruses are subjected to less selective pressure. *Tobacco mosaic virus* (TMV), an RNA virus from the genus *Tobamovirus*, is able to infect a wide range of hosts, including *Arabidopsis thaliana*. Additionally, various *Arabidopsis* ecotypes exhibit diverse responses to TMV. For instance, Shahdara (Sha) is susceptible, exhibiting a high viral accumulation early on, with a distinct leaf curling symptom; Tsu-0 is resistant, with minimal viral accumulation; and Col-0 is tolerant, with substantial viral accumulation but no visible symptoms (1). In the GreenTolerance project, our first goal was to characterize the tolerance phenotype across the Sha, Col-0, and Tsu-0 ecotypes. We measured symptoms, viral load and fitness traits (including seed set, biomass, stalk length, rosette diameter), and conducted multiomics analysis through transcriptomics and untargeted metabolomics measures. Sha, Col-0 and Tsu-0 ecotypes exhibited differences in symptom, viral load, fitness traits, and metabolomic and transcriptomic responses to the virus, highlighting the specificities of the susceptible, tolerant and resistant responses. These specificities allowed us to identify tolerance markers, including a quantitative measure of tolerance called "range tolerance" (2). We used these markers to perform a genome-wide association study (GWAS) on 135 *Arabidopsis thaliana* ecotypes from a worldwide collection. These ecotypes displayed a wide range of tolerance levels, while only Shahdara had visible symptoms with the highest viral accumulation. The GWAS analysis allowed the identification of candidate genes for TMV resistance and a distinct set of genes for tolerance. Further experiments will be conducted to confirm the involvement of these candidate genes in tolerance.

Mots clés : Tolerance - Virus - Plant - Genetic.

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C-30

Does multiple infection of tomato leaf curl New Delhi virus (ToLCNDV) and watermelon mosaic virus (WMV) cause a more severe disease in melon?

Atiwich Patthamapornsirikul (atiwich.patthamapornsirikul@inrae.fr), Eric Verdin, Cécile Desbiez

Pathologie Végétale, INRAE, Montfavet, France

The emergence of Tomato leaf curl New Delhi virus (ToLCNDV; *Begomovirus solanumdelhiense*) in France and across the Mediterranean can induce complex interactions with other cucurbit-infecting viruses present in the area, leading to varied outcomes in disease severity and viral accumulation. These interactions can present distinct types, including synergism, where viruses mutually enhance each other's effects; asymmetric synergism, where one virus or viral component in a multipartite virus gains disproportionately over the other; and antagonism, where one virus restricts the other's impact. ToLCNDV has previously demonstrated asymmetric synergistic interactions with other begomoviruses and antagonistic relationships with certain tobamoviruses. In France, where ToLCNDV was first detected in 2020, some isolates presented an atypical "recover" phenotype, where symptom severity in susceptible cucurbits decreased in the course of infection. We studied the potential interactions of ToLCNDV with watermelon mosaic virus (WMV; *Potyvirus citrulli*), a prevalent virus in Southern France. A severe Spanish isolate ES13-35 and a French "recover" isolate FR443-1, were co-transmitted with WMV in mixed-sap inoculations on susceptible melon plants. Physical measurements—such as disease index, plant height, and weight—were used to assess disease severity, while viral accumulation was measured using multiplex TaqMan qPCR. Mixed infections produced more severe symptoms than single infections, and the recovery associated to isolate FR443-1 was no more observed, Mixed infection resulted in increased ToLCNDV accumulation without change in WMV accumulation, suggesting an asymmetric synergistic relationship where ToLCNDV gains disproportionately, potentially exacerbating disease impacts on cucurbit crops. This study highlights the need for targeted management strategies to mitigate these complex viral interactions in Mediterranean agriculture.

Mots clés : ToLCNDV - WMV - Mixed infection - Virus interactions - Synergism.

C-31

Making use of an axillary component from the multipartite nanovirus for virus-induced silencing of insect vector genes

Auguste Rajot[1] (auguste.rajot@gmail.com), Bérénice Reggiardo[2], Nathalie Laboureau[3], Stéphane Blanc[4], Mikhail M. Pooggin[4]

[1] *PHIM Plant Health Institute of Montpellier, Université de Montpellier, Montpellier, France*
[2] *Université de Montpellier, Montpellier, France*
[3] *PHIM, CIRAD, Montpellier, France*
[4] *PHIM, INRAE, Montpellier, France*

Faba bean necrotic stunt virus (FBNSV) is a multipartite nanovirus (family *Nanoviridae*) that infects legumes causing severe symptoms. The virus consists of eight single-stranded DNA components, each encoding one single protein: R (replication initiator), S (capsid), M (movement), N (nuclear shuttle), C (cell cycle link), U1, U2, and U4 (unknown functions). U4 was reported to be dispensable for viral replication, symptom development and plant to plant transmission by the aphid vector under laboratory conditions, making this component a good candidate for a virus-induced gene silencing (VIGS) approach. In this approach the U4 sequence is modified by short or long inserts of candidate aphid target genes. The insert-derived small interfering RNAs generated by the antiviral RNA interference machinery in virus- and VIGS construct-infected plants are expected to move systemically via phloem system and to be ingested by the aphids feeding on phloem sap to direct silencing of the aphid target gene. In proof-of-concept experiments, we modified the U4 sequence to insert fragments of two aphid genes: C002, an effector-encoding gene required for aphid feeding, and SCAMP1 implicated in virus-aphid interactions. We found that the modification of only 44 nucleotides in the 3' untranslated region of U4 mRNA reduced U4 DNA accumulation in systemically-infected leaves, while deletion of the U4 protein-coding sequence abolished U4 systemic movement. However, despite the reduced accumulation of the modified U4 component in systemically infected upper leaves, we observed notable effects on virus transmission efficiency, especially in the case of C002 inserts replacing the U4 ORF. These findings suggest that U4-based VIGS vectors can be used for silencing aphid genes, even with limited systemic spread. Surprisingly, deletion of the U4 protein-coding sequence also impacted virus transmission, albeit less-pronouncedly, suggesting that U4 protein may play a role in virus transmission.

Mots clés : FBNSV - VIGS - Aphid - Transmission - U4.

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C-32

Datamining public databases in search of grapevine viruses

Wassim Rhalloussi[1, 2] (wassim.rhalloussi@inrae.fr), Jean-Michel Hily[2, 3]

[1] *UMR-A 1131 Santé de la Vigne et Qualité du Vin, INRAE, Université de Strasbourg, Colmar, France*
[2] *Laboratoire Partenarial Associé Vitivirobiome, Colmar, France*
[3] *Institut Français de la Vigne et du Vin, Le Grau-du-Roi, France*

With the dawn of high throughput sequencing (HTS), the submission and accumulation of genetic information within dedicated databases (datasets) is substantial and ever growing. So far, the interest being the host's sequences, the data analyzed corresponds only to the tip of the iceberg and an important portion of it goes unchecked. Datamining is the process of collecting, searching, extracting and discovering usable information within such large amount of data. Our project, based on datamining, focuses on most (if not all) publicly available datasets originating from plants of the *Vitis* genus, in an attempt to reconstruct viral genomes and identify the virome of each dataset.
To ensure reproducibility and automation of multiple analyses, we first developed a custom inhouse *Nextflow* pipeline that uses commonly available bioinformatic tools to assemble primarily viral genomes or contigs, but also other pathogens, and then identifies them with *BLAST* against *nt* or *nr* database. Using NCBI's *Sequences Read Archive (SRA) explorer*, we selected all publicly available runs of sequencing originating from *Vitis* samples and retrieved their metadata table. We used this table to filter out non-informative datasets, such as amplicon sequencing-based data, to keep only RNA-seq and small-RNA data. Each dataset is downloaded from the *European Nucleotide Archive's (ENA)* and fed to the pipeline.
As of august 2024, a total of 17,874 runs were available on NCBI's *SRA*. To this day, a subset of 573 datasets have been analyzed, generating 532,734 contigs of 500 bp or longer. Of these, 6,466 were identified as grapevine viruses. These viral genomes, assembled by the pipeline, will allow us to conduct further analysis: from the design of new and more exhaustive detection primers considering the genetic diversity (new variants and SNPs) of the targeted viruses; up to the potential reconstruction of the evolutive history of some viral species.

Mots clés : Datamining - High throughput sequencing (HTS) - Pipeline - Virus - Grapevine.

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C-33

Unexpected virus reservoirs in vegetatively propagated plants: A case study of sweetpotato

Estel Pakyendou Name[1, 2, 3] (pakyendou.name@gmail.com), Fidèle Tiendrebeogo[2, 3], Ezechiel Bionimian Tibiri[1, 2, 3], Seydou Sawadogo[1, 2, 3], Justin Pita[3]

[1] *Laboratoire de Virologie et de Biotechnologie végétale, Institut de l'Environnement et de la Recherche Agricole (INERA), Ouagadougou, Burkina Faso*
[2] *Laboratoire de Biologie moléculaire et de Génétique (LABIOGENE), Université Joseph Ki-Zerbo, Ouagadougou, Burkina Faso*
[3] *Central and West African Virus Epidemiology (WAVE) for Food Security Program, Pôle Scientifique et d'Innovation, Université Félix Houphouët-Boigny (UFHB), Abidjan, Côte d'Ivoire*

Sweetpotato (*Ipomoea batatas*) is a significant global food crop, widely cultivated in tropical and subtropical regions due to its nutritional benefits. However, its production is severely affected by a variety of viral diseases, including *Sweet potato feathery mottle virus* (SPFMV), *Sweet potato chlorotic stunt virus* (SPCSV), and *Sweet potato leaf curl virus* (SPLCV), which contribute to substantial yield losses. This study focuses on the analysis of circular single-stranded DNA viruses infecting sweetpotato in Burkina Faso. A total of 52 sweetpotato leaf samples, showingtypical symptoms such as yellowing, mosaic, curling, and leaf deformation, were selected from a larger set of samples collected during a previous study conducted between 2015 and 2016 from various production areas. Total DNA was extracted using the CTAB protocol, followed by Oxford Nanopore sequencing with the Native Barcoding Kit (SQK-NBD114.96), in combination with *rolling circle amplification*. Bioinformatics analysis revealed the presence of *Sweet potato leaf curl virus* (SPLCV) and *Sweet potato leaf curl deltasatellite 3* (SPLCD3). In addition, non-native viruses, including *Pepper yellow vein Mali virus* (PepYVMV; 2781 kb), *Cotton leaf curl Gezira alphasatellite* (CLCuGeA; 1353 kb) and *Cotton leaf curl Gezira betasatellite*(CLCuGeB; 1348 kb), were identified. These findings represent the first report of PepYVMV, CLCuGeA, and CLCuGeB in association with sweetpotato virus diseases, both in Burkina Faso and globally. The results suggest that sweetpotato may serve as a potential reservoir for diverse viral pathogens, with implications for the management of viral transmission dynamics and the persistence of these viruses in agricultural systems.

Mots clés : Alternative hosts - Nanopore sequencing - Sweetpotato - Virus reservoirs - Viral diversity.

C-34

MED18: a bridge for cauliflower mosaic virus mRNAs export

Anita Kaltak (anita.kaltak@etu.unistra.fr), Mattia Dall'Ara, Jón Pol Gales, Maria Dimitrova

Institut de Biologie Moléculaire des Plantes (IBMP), Strasbourg, France

Cauliflower mosaic (pararetro)virus is transmitted to plant cells by aphids and is transported to the nuclear pores through which the viral genome is released into the nucleus. The genome is then repaired, condensed into a mini-chromosome and transcribed into two mRNAs, the monocistronic 19S and the polycistronic and alternatively spliced 35S RNA. The viral RNAs are exported to the cytoplasm in order to be translated and/or reverse-transcribed. We have recently demonstrated that several host and viral factors participate in the nuclear export of CaMV 35S RNA: adaptor proteins MOS and ALY, the P4 coat protein and reverse transcriptase P5, as well as the highly structured 5' leader region which loads these export factors (Kubina *et al.,* 2021).
To identify additional cellular proteins that interact with the 5' leader region and promote 35S RNA nuclear export, we performed *in vitro* affinity purification coupled to mass spectrometry. This approach allowed the identification of several Arabidopsis nuclear proteins including MED18, a subunit of the MEDIATOR complex, and NUA, a protein of the nuclear basket. *Med18* mutant Arabidopsis plants are resistant to CaMV infection (Hussein *et al*., 2020), and we demonstrated that 35S RNA nuclear export is strongly inhibited, both in *med18* and *nua* mutant protoplasts. Moreover, we discovered that MED18 also interacts with viral protein P4 and with several subunits of the TREX2 export complex located at the nuclear pore. Thus, MED18 likely acts as an export factor bridging the viral mRNPs at the nuclear pore and promoting their export.

Mots clés : CaMV - mRNA export - MED18 - NUA - 35S RNA.

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C-35

Identifying the impact of insecticide resistant grain aphids (*Sitobion avenae*) on barley yellow dwarf virus epidemiology in Ireland

Maximilian Schughart[1] (maximilian.schughart@teagasc.ie), Emmanuel Jacquot[2], Louise McNamara[1], Stephen Byrne[1]

[1] *Teagasc Crop Science, Teagasc Ireland, Carlow, Irlande*
[2] *Plant Health Institute Montpellier, INRAE/CIRAD, Montpellier, France*

Resistance to pyrethroids in the English grain aphid (*Sitobion avenae*) has spread throughout Europe, including Ireland, since 2013. *S. avenae* that survive spray applications can cause damage to crops by feeding on the grain and by transmitting barley yellow dwarf viruses (BYDV). The 2018 EU-ban on neonicotinoids leaves pyrethroid applications as the remaining control option, and it has therefore been hypothesized that BYDV pressure will increase, because of the favourable selection of pyrethroid resistant *S. avenae* after spray applications. Given the potential agricultural impact, a three-year field study was conducted. The findings revealed a decline in the occurrence of insecticide-resistant *S. avenae,* and no link between aphid resistance and high BYDV levels was found. Furthermore, BYDV transmission experiments demonstrated that the resistant *S. avenae* clonal lineage is not a better BYDV transmitter than other susceptible *S. avenae* clones. However, virus-aphid behavioural manipulation experiments showed that BYDV-infected aphids exhibit increased movement activity, and that different BYDV species manipulate aphid behaviour in different ways. The results underscore the complex tripartite interactions between aphids, viruses, and crops, all of which influence BYDV epidemiology. This should be taken into account when developing future decision support tools.

Mots clés : BYDV - Aphids - Crop Protection - Epidemiology - Insecticide resistance.

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Development of polerovirus-induced gene silencing for rapid and visual screening in sugar beets

Heemee Devi Bunwaree[1] (heemee-devi.bunwaree@etu.unistra.fr), Elodie Klein[2], Guillaume Saubeau[2], Bruno Desprez[2], Véronique Ziegler-Graff[1], David Gilmer[1]

[1] *Institut de Biologie moléculaire des Plantes, Strasbourg, France*
[2] *Florimond Desprez, Cappelle-en-Pévèle, France*

Since the ban of neonicotinoid insecticides in the European Union, sugar beet production is threatened by outbreaks of virus yellows (VY) disease, caused by several aphid-transmitted viruses, including the polerovirus beet mild yellowing virus (BMYV). As the symptoms induced may vary depending on multiple infections and other stresses, there is an urgent need of fast screening tests to evaluate resistance/tolerance traits in sugar beet accessions. To respond to this issue, we exploited the virus-induced gene silencing (VIGS) system to produce a functional recombinant BMYV able to induce evident vein clearing symptoms in infected sugar beets. By introducing a fragment of an endogenous *Beta vulgaris* gene involved in chlorophyll synthesis in the BMYV genome, we produced a recombinant BMYV, capable of generating clear vein chlorosis symptoms in infected sugar beets, as early as ten days post agroinoculation, allowing easy and rapid visual discernment of infected plants, across five different sugar beet lines. The recombinant virus displayed similar infectivity as the wild-type virus, and the insert remained stable within the viral progeny. Our results also indicated that the percentage of VIGS-symptomatic plants was representative of the infection rate of each evaluated line. To optimise the system to high-throughput screenings, a time course experiment was conducted over eight weeks and a time lag between viral infection and development of VIGS symptoms was observed. By analysing viral RNAs and small interfering RNAs (siRNAs) in infected plants, we hypothesised that a prerequisite threshold of viral RNAs is required for VIGS symptoms to appear. Finally, we demonstrated that depending on the susceptibility of the line to BMYV infection, VIGS symptoms may last over months. Thus, our work provides a polerovirus based VIGS system adapted to sugar beet crop allowing visual and rapid large-scale screens for resistance or functional genomic studies.

Mots clés : Polerovirus - Beet mild yellowing virus - Beta vulgaris - Viral yellows - Viral induced gene silencing.

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C-37

Discovery and biological characterization of a novel emaravirus infecting blackberry in Belgium

Pierre Hellin[1] (p.hellin@cra.wallonie.be), Pauline Richet[1], Elisabeth Demonty[1], Nathalie Dubuis[2], Arnaud G. Blouin[2], Stéphan Steyer[1], Sébastien Massart[3]

[1] *Santé des Plantes et Forêts, Centre Wallon de Recherche Agronomique (CRA-W), Gembloux, Belgique*
[2] *Virologie, Bactériologie et Phytoplasmologie, Agroscope, Nyon, Suisse*
[3] *Gestion durable des bio-agresseurs, Gembloux Agro-Bio Tech (Ulg), Gembloux, Belgique*

A blackberry plant (*Rubus fructicosus*) from a Belgian plant nursery presenting leaf blotch and vein banding/feathering symptoms was analyzed by high throughput sequencing of ribodepleted total RNA. Multiple RNA fragments belonging to a potential emaravirus (family *Fimoviridae*) were detected. Moreover, spherical bodies morphologically similar to emaravirus virions observed by electron microscopy strengthened this finding. The full genomic sequence, acquired by Sanger sequencing, indicated that the virus possessed the four core segments typical of emaravirus as well as three additional RNAs. The proteins encoded by the core RNA segments were all at least 20% different from all known emaravirus protein sequences, supporting its classification as a new species. The virus appeared to be most closely related to raspberry leaf blotch virus (RLBV aka *Emaravirus idaeobati*) infecting raspberry (*R. ideaus*) in the EU, and was distant from blackberry leaf mottle-associated virus (BLMaV aka *Emaravirus rubi*), infecting blackberry in the USA. The novel virus was therefore provisionally named blackberry leaf blotch virus (BLBV). A small survey was performed on bramble (wild *Rubus*), presenting the same typical symptoms observed on the initial blackberry plant. It highlighted the presence of BLBV in three other locations in Belgium, with the new isolates showing high protein homology with the reference isolate. In addition, an Eriophyid mite (typical vector of emaravirus) was found on both the blackberry plant and wild bramble. Molecular barcoding indicated that this mite was closely related to *Phyllocoptes gracillis*, the vector of RLBV on raspberry, yet might also belong to a novel species. Transmission bioassays performed in greenhouse on blackberry suggested that it is likely the vector of BLBV. This discovery urges the question of the impact of BLBV on blackberry crops and reaffirms the need for virus surveillance in plant propagation and importation as well as the use of certified plants.

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RÉSUMÉS DES COMMUNICATIONS

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C-38

Dispersion and evolutionary history of rice yellow mottle virus in Africa: tales of rice and men

Nils Poulicard[1] (nils.poulicard@ird.fr), Oumar Traoré[2], Souley Issaka[3], Agnès Pinel-Galzi[1], Jamel Aribi[1], Aurore Comte[1], Edgar Traoré[2], Yacouba Séré[4], Drissa Silué[4], Eugénie Hébrard[1], Denis Fargette[1]

[1] *PHIM, Université Montpellier, IRD, INRAE, Cirad, Institut Agro, IRD, Montpellier, France*
[2] *INERA, LNB, Ouagadougou, Burkina Faso*
[3] *Université Boubakar Bâ, FSA, Tillabéri, Niger*
[4] *AfricaRice, Bouaké, Côte d'Ivoire*

Rice has become a pillar of food security in Africa. During the 20th century, rice cultivation intensified to cope with the rising demand due to demographic changes in Africa. Rice yellow mottle virus (RYMV, *Solemoviridae*) is a major biotic constraint to rice production in Africa. RYMV is a (+)ssRNA virus transmitted at short distances by beetles and by contact between plants during cultural practices. There is no evidence of seed transmission. Seven major strains have been identified with a marked spatial diversity. Several sources of resistance to RYMV have been identified in rice, but none are currently widely used in field. Resistance-breaking risk maps have been proposed based on the spatial distribution of the strains and their pathogeny estimated under controlled conditions. However, the validity and the sustainability of these risk maps are strictly dependent on the dispersal and adaptive characteristics of the RYMV in field conditions.
The main objectives of this study are i) to reconstruct the dispersal dynamics of RYMV in Africa, ii) to identify the main drivers of RYMV evolution and dispersal, and iii) to estimate the impact of RYMV evolutionary history on the sustainability of resistance genes in fields.
Based on RYMV genetic data collected in Africa since the 1970s, the phylogeography of RYMV was reconstructed using Bayesian evolutionary inference. These spatiotemporal reconstructions revealed links between RYMV expansion dynamics, the evolution of rice production in Africa and the migration of human populations during historical conflicts. Furthermore, we showed that the spatial dispersal of the RYMV has shaped its genetic evolution, with the emergence of adaptive mutations to new host species and interstrain recombination events.
Overall, combining field epidemiology, experimental assays and modelling, we have partially unravelled the balance and interplay between genetic determinants and stochasticity in the evolution and epidemiology of a plant virus.

Mots clés : Phylogeography - Phylodynamics - Experimental evolution - Host adaptation - Resistance-breaking.

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C-39

Role of a short non coding viral sequence in bypassing crossprotection in tomato infecting begomoviruses

Cica Urbino (cica.urbino@cirad.fr), Margaux Jammes, Julie Balland, Joaquim Celuzza, Martine Granier, Stéphane Blanc, Pascal Alonso, Michel Peterschmitt

PHIM, CIRAD, Montpellier, France

TYLCV-IS76 (IS76) is a natural recombinant of Tomato Yellow Curl Virus (TYLCV, *Begomovirus, Geminiviridae*) in which 76 nucleotides (nts) of the intergenic region (S76) have been replaced by the homologous sequence from Tomato Yellow Curl Sardinia Virus (TYLCSaV). We monitored the emergence of IS76 in Morocco and showed, in controlled conditions, that its intra-plant accumulation was significantly higher than those of parental viruses in resistant cultivars carrying the *Ty-1* resistance gene. This gene is known to code for a γ-clade RNA-dependent RNA polymerase that prevents symptoms and reduces viral load. The competitive advantage of IS76 is detected irrespective of co-infection conditions, simultaneously with parents or 1 or 4 months after parents, which questioned the existence of crossprotection with TYLCV and more generally with begomoviruses.
Using TYLCV variants differing by 8 or 30 nts within the S76 region and qPCR monitoring of viral DNA accumulations, we proved the existence of a crossprotection phenomenon with the TYLCV parent, in Ty-1-resistant and susceptible tomato plants, and in turn that IS76 escapes this mechanism. Although crossprotection mechanisms with TYLCV are not yet known, we studied the genetic determinism of the crossprotection-escape and more specifically whether it is determined only by the S76 region. It this is true, the escape would be observed with the TYLCSaV parent, the donor of S76, and also with any other begomovirus that inherit S76 by recombination. A TYLCSaV mutant and a recombinant Tomato leaf curl Comoros virus (ToLCKMV) carrying S76 were engineered to test this hypothesis. Results will be discussed in relation with the emergence of IS76 and more generally with the crossprotection phenomenon in begomoviruses and its potential application in their management.

Mots clés : Premunition - Viral accumulation - Emergence - TYLCSaV.

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C-40

Insights into mature plant resistance in sugar beet and inhibition of virus transmission by aphids

Véronique Brault (veronique.brault@inrae.fr), Fanny Siffert, Anabella Heintz, Hélène Schlaefli, Alessandra Maia-Grondard, Aurélie Marmonier, Quentin Chesnais, Martin Drucker

UMR1131 SVQV, INRAE, Colmar, France

Several plant species acquire an increasing degree of resistance against pathogens, or insects, during their development, which is referred as to Mature Plant Resistance (MPR). The molecular mechanisms sustaining MPR are not fully understood, and are probably multifactorial. MPR of sugar beet is effective when the plant reaches 10 to 14 fully developed leaves and is exemplified by appearance of a black deposit in the aphid's stomach shortly followed by death. MPR was shown to vary depending on sugar beet genotypes1 and can be lowered by some viruses2.

Sugar beet can be infected by four viruses causing leaf yellowing that are transmitted by aphids but using different modes. We have observed that the MPR of sugar beet was efficient enough to inhibit virus transmission of beet chlorosis virus (BChV) and beet mild yellowing virus (BMYV), two poleroviruses transmitted in a circulative and persistent manner. In opposite, sugar beet MPR did not inhibit aphid transmission of beet yellows virus (BYV, *Closterovirus*, semi-persistent transmission), or beet mosaic virus (*Potyvirus*, non-persistent virus). The sugar beet resistance to BChV inoculation was further characterized by analyzing the feeding behavior of aphids by electropenetrography (EPG) on sugar beet at different stages of development. EPG analyses did not reveal any antixenosis effects that could explain the observed reduction in poleroviruses transmission. A metabolomic analysis was carried out during sugar beet development (3 to 7 weeks post-sowing) and at different leaf stage levels (young/mature leaves) to identify changes correlating with MPR establishment. Specific metabolites signatures were observed in leaves of old plants which could be linked to aphid antibiosis. The link between the over-accumulation of some compounds and aphid's survival rate is currently being assessed. Identification of such markers for MPR could be exploited for breeding sugar beet genotypes likely to limit impact of aphid-transmitted viruses.

Mots clés : Sugar beet viruses - Aphid transmission - Mature plant resistance - Metabolomic analysis - Aphid feeding behavior.

Références :

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2. Kift et al., 1996. The effect of plant age and infection with virus yellows on the survival of Myzus persicae on sugar beet. Annals of Applied Biology. https://doi.org/10.1111/j.1744-7348.1996.tb05761.x

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Uncovering the genetic basis of quantitative resistance to cucumber mosaic virus in *Capsicum annuum* using genome-wide association coupled with high-throughput image analysis

Judith Hirsch[1] (judith.hirsch@inrae.fr), Lucie Tamisier[2], Marion Szadkowski[1], Sylvain Piry[1, 3], Louis McLeod[2, 4], Anne Massire[2], Christophe Gros[2], Benoît Moury[1], Véronique Lefebvre[2]

[1] *Pathologie végétale, INRAE, Montfavet, France*
[2] *GAFL, INRAE, Montfavet, France*
[3] *CBGP, INRAE, Montpellier, France*
[4] *A2M, INRAE, Montfavet, France*

Using cultivars with genetic resistance to viruses is a key strategy for controlling viral epidemics in crops. Quantitative resistance is likely to be more durable than qualitative resistance and can further enhance the durability of major-effect resistance genes when combined in a pyramiding strategy. Thus, understanding the genetic basis of quantitative resistance to viruses holds significant promise for improving resistance durability in breeding programs. We addressed this question in the case of *Capsicum annuum* resistance to cucumber mosaic virus (CMV), an economically important virus in pepper crops worldwide. We focused on the early stages of CMV infection to identify resistance mechanisms that occur rapidly during the infection process. A panel of 336 *C. annuum* accessions from the G2P-SOL H2020 core-collection (McLeod et al., 2023) was inoculated with the N strain of CMV, which induces local lesions. Symptom severity, assessed by the extent of necrotic lesions on inoculated leaves (ten leaves per accession), was analyzed through a deep learning pipeline. Phenotypic and genotypic data were then combined in a genome-wide association study (GWAS) using mixed linear and multi-locus mixed models.
The deep learning pipeline achieved over 98% accuracy in detecting and classifying necrotic lesions, demonstrating its effectiveness as a high-throughput phenotyping tool. GWAS identified SNPs significantly linked to resistance, including a major SNP on chromosome 6 consistently detected using both models. Additional significant loci were found on chromosomes 1, 8, and 12. Notably, the region on chromosome 6 has previously been associated with resistance to potato virus Y, a phylogenetically unrelated virus.
This study illustrates the power of combining GWAS with deep learning phenotyping to accelerate resistance breeding. Future research will explore candidate genes underlying the identified Quantitative Trait Loci (QTL), particularly those associated with the QTL on chromosome 6, which may provide insights into early broad-spectrum virus resistance mechanisms.

Mots clés : Capsicum annuum - Cucumber mosaic virus - Quantitative resistance - Durability - GWAS.

Références :

1. McLeod et al. 2023, Plant J. 116, 1508–1528.

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C-42

Plasma membrane-localized lipids are involved in plant response during infection by *Potexviruses*

Emeline Teyssier[1] (emeline.teyssier@u-bordeaux.fr), Brian Vue[1], Jahed Ahmed[1], Véronique Germain[1], Sébastien Montgrand[2]

[1] *Laboratoire de Biogenèse Membranaire, Université de Bordeaux, Villenave-d'Ornon, France*
[2] *Laboratoire de Biogenèse Membranaire, CNRS, Villenave-d'Ornon, France*

Plant antiviral responses rely on RNA interference and Effector-Triggered Immunity, which mainly involve cytosolic processes. However, an increasing number of reports suggest that the plasma membrane (PM) could also play a role. Whereas most studies until now focused on PM proteins, we have investigated the role of PM lipids, as recent data suggest they also contribute to plant immunity. An *Arabidopsis thaliana* mutant, specifically affected in the biosynthesis of PM-localized sphingolipids, was selected and its susceptibility toward a *Potexvirus*, the *Plantago asiatica Mosaic Virus* (PlAMV) was characterized. PlAMV propagation was hampered in the mutant line both locally and systemically, revealing a role for sphingolipids during plant infection by *Potexvirus*. Their localization at the PM suggest that sphingolipids could be involved in signaling pathways linking virus perception and plant defense responses. We have explored the hypothesis of a link between the role of sphingolipids during virus infection and the production of apoplastic ROS. In Arabidopsis leaves, two PM-localized NADPH oxydases, Respiratory Burst Homologs D (RBOHD) and F (RBOHF) are responsible for the production of ROS in the apoplasm. These enzymes generate superoxide that spontaneously dismutate into hydrogen peroxide (H_2O_2). Interestingly, PlAMV spreading was enhanced in both *rbohD* and *rbohF* single mutant, demonstrating that apoplasm ROS production participate in plant defense during PlAMV infection. In order to decipher the underlying mechanisms, we have measured H_2O_2 in control and infected leaves with high spatial resolution, using a genetically encoded H_2O_2 reporter, HyPer7 addressed to the cytosolic leaflet of the plasma membrane. In the WT, Hyper7 revealed variable H_2O_2 contents depending on the presence/absence of the virus and on the infection spatial progression. Moreover, in the sphingolipid mutant, these variations were not observed, suggesting that the modification of the PM sphingolipids could impair H_2O_2-related signaling at the plasma membrane and consequently alter PlAMV propagation.

Mots clés : Sphingolipid - Plasma membrane - Potexvirus - H2O2.

C-43

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

Khalid Amari (khalid.amari@julius-kuehn.de), Mohammad Vatanparast, Lisa Merkel

Julius Kühn Institute, Quedlinburg, Germany

RNA interference (RNAi) is a biological process that regulates gene expression and protein synthesis by targeting specific mRNA sequences. In plants, the exogenous application of double-stranded RNA (dsRNA) can induce systemic gene silencing, leading to the suppression of specific genes in different tissues and organs. This technique has gained attention as an effective plant protection strategy against various pathogens, including viruses, fungi, and insect pests. The use of formulated dsRNA has proven to be highly effective against a wide range of pathogens and pests. When it comes to viruses, the application of formulated dsRNA has been found to significantly enhance dsRNA stability for a long period, offering robust protection against viral infections. However, the use of dsRNA in plant protection raises several safety concerns, such as off-target effects, effect on non-target organisms, epigenetics, and immune system activation. Here, we will discuss opportunities, challenges and risk assessment of dsRNA-based plant protection products.

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Effect of mutations in intrinsically-disordered or ordered regions of potato virus Y (PVY ; genus *Potyvirus* ; family Potyviridae) VPg on virus fitness and adaptability

Guillaume Dubrulle[1] (guillaume.dubrulle@univ-reims.fr), Guillaume Lafforgue[2], Justine Charon[2], Amandine Barra[2], Marion Szadkowski[1], Lola Chateau[1], Cécile Desbiez[1], Thierry Michon[2], Benoit Moury[1]

[1] *Pathologie végétale, INRAE, Montfavet, France*
[2] *UMR Biologie du Fruit et Pathologie, INRAE, Université de Bordeaux, Villenave-d'Ornon, France*

Viruses display a particularly high number of intrinsically-disordered regions (IDR) within their proteins. Intrinsic disorder can be beneficial to the fitness and evolutionary potential of viruses. We have previously shown (Charon et al. 2018) that mutations in the VPg of PVY that increase the level of intrinsic disorder have a positive effect on PVY adaptability in pepper and that a mutation that decreases the level of intrinsic disorder has a negative effect on PVY adaptability. However, few mutations were tested and only one VPg domain was mutated, with a dual function affecting both intrinsic disorder and interaction with the plant resistance factor.
Here, we tested whether random mutations spanning the entire VPg differ in their effect on PVY fitness and adaptability, depending on whether they occur in the intrinsically disordered or ordered regions of the VPg. To test this hypothesis, we will present, for 15 PVY mutants, (i) measures of intra-plant accumulation, symptoms and impact of infection on plant weight in the susceptible pepper genotype Yolo Wonder and (ii) infection efficiencies of pepper genotype HD285, carrying resistance gene *pvr23* (as a measure of adaptability). Of these 15 mutants, only one showed a higher accumulation than the wild-type PVY strain, and only two had a higher adaptability. Fourteen mutants induced a lower reduction of plant weight than the wild-type.
Globally, these data do not support the hypothesis that mutations in intrinsically-disordered regions increase virus fitness or adaptability.

Mots clés : Intrinsic disorder - Protein structure - Fitness - Evolvability.

Références :

1. Charon J, Barra A, Walter J, Millot P, Hébrard E, Moury B, Michon T. 2018. First Experimental Assessment of Protein Intrinsic Disorder Involvement in an RNA Virus Natural Adaptive Process. Mol Biol Evol 35:38–49.

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C-45

À la recherche des réservoirs des virus responsables des jaunisses de la betterave

Thierry Candresse[1] (thierry.candresse@inrae.fr), Armelle Marais[1], Amélie Monteiro[2], Yannis Nio[3], Bernard Bergey[1], Laurence Svanella-Dumas[1], Fabienne Maupas[2], Jean Christophe Simon[3], Véronique Brault[4]

[1] *UMR 1332 BFP, INRAE, Villenave-d'Ornon, France*
[2] *ITB, Paris, France*
[3] *UMR IGEPP, INRAE, Le Rheu, France*
[4] *UMR SVQV, INRAE, Colmar, France*

L'interdiction des néonicotinoïdes a augmenté l'impact des viroses transmises par pucerons pour de nombreuses grandes cultures. Chez la betterave, quatre virus ayant différents modes de transmission par pucerons causent des jaunisses : beet yellows virus (BYV, Closterovirus), beet chlorosis virus and beet mild yellowing virus (BChV and BMYV, Polerovirus) and beet mosaic virus (BtMV, Potyvirus). Malheureusement, nous manquons encore d'informations clés sur la biologie de ces virus comme l'identité de leur(s) réservoir(s) ou leurs distances de dispersion par pucerons. Nous avons utilisé la métagénomique virale dsRNA pour tenter d'identifier ces réservoir(s). Des adventices et des espèces cultivées ont été échantillonnées dans cinq régions betteravières de France au printemps 2023, en focalisant sur des espèces connues comme hôtes expérimentaux des virus des jaunisses. Le virome de la betterave sauvage (*Beta maritima*) a également été analysé.
L'analyse du virome de plus de 4000 plantes apporte quelques conclusions importantes : (1) parmi les 18 espèces végétales analysées seules la Phacélie (*Phacelia tanacetifolia*) et la fèverole apparaissent comme des hôtes naturels de deux des virus étudiés (BtMV et BYV); (2) les betteraves sauvages sont fréquemment infectées par le BtMV et/ou le BYV (7/9 populations) mais contrairement à la Phacélie, les isolats de betterave sauvage sont différents de ceux de betterave cultivée, traduisant l'existence de barrière(s) aux flux viraux. La Phacélie est une plante mellifère largement utilisée en interculture. La mise en évidence d'un rôle de réservoir potentiel a conduit à des recommandations de gestion. Aucun réservoir potentiel n'a été identifié pour le BChV et le BMYV, suggérant un possible rôle des betteraves résiduelles en post-culture. Par contre, le turnip yellows virus, principal polérovirus du colza, a été détecté chez 10/18 espèces testées, indiquant une épidémiologie vraisemblablement très différente en matière de réservoir entre des polérovirus de betterave et de colza par ailleurs très proches.

Mots clés : Virome - REservoir - Jaunisses - Betterave - Flux viraux.

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RÉSUMÉS DES COMMUNICATIONS AFFICHÉES

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P-01

Variability of rice gene silencing defense mechanism against different rice infecting viruses

Aurore Comte (aurore.comte@ird.fr), Nils Poulicard, Martine Bangratz, Séverine Lacombe

UMR PHIM, IRD, Montpellier, France

Gene silencing acts as an antiviral mechanism through the synthesis of viral small interfering RNA (vir-siRNA). RNA viruses mainly induce the dcl4 gene silencing mechanism, which directs specific viral RNA degradation mediated by 21 nt vir-siRNA, whereas DNA viruses also induce the dcl3 gene silencing mechanism, which produces 24 nt vir-siRNA that directs repression of viral transcription through DNA methylation. To investigate the variability of these gene silencing responses to different viruses within the same host, six different rice viruses identified in Africa and/or South America were considered for deep sequencing of the small RNA population of infected rice leaves. Already known rice RNA viruses such as rice yellow mottle virus (RYMV, *Sobemoviridae*), rice stripe necrosis virus (RSNV, *Benyviridae*) and rice hoja blanca virus (RHBV, *Phenuiviridae*) were considered. Maize streak virus (MSV, *Geminiviridae*), recently identified for the first time in rice, was also studied. In addition, two undescribed RNA viruses belonging to the families *Potyviridae* or *Rhabdoviridae* identified in Africa were considered. All these RNA or DNA viruses showed variable genomic organization, ranging from monopartite to quadrupartite. Bioinformatic analyses of small RNA libraries were performed to determine the size distribution of vir-siRNA and these observations were compared with the expected 21 nt / 24 nt vir-siRNA distribution for RNA and DNA viruses, respectively. Vir-siRNA distributions on viral genomes and the proportion of vir-siRNA in total small RNA were evaluated. All these data have helped to refine the knowledge of the variability of the gene silencing defense mechanism against different viruses within the same host, in this case rice.

Mots clés : Gene silencing - Rice viruses - Viral siRNA.

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P-02

Improving Cassava Mosaic Disease detection through Oxford Nanopore Technologies Approach on cassava leave in Burkina Faso

Seydou Sawadogo[1, 2, 3] (seydousawadogo66@gmail.com), Fidèle Tiendrebeogo[2, 3], Ezechiel Bionimian Tibiri[1, 2, 3], Estel Pakyendou Name[1, 2, 3], Justin Pita[3]

[1] *Laboratoire de Virologie et de Biotechnologies Végétales, Institut de l'Environnement et de Recherches Agricoles (INERA), Ouagadougou, Burkina Faso*
[2] *Laboratoire de Biologie Moléculaire et de Génétique (LABIOGENE), Université Joseph Ki-Zerbo, Ouagadougou, Burkina Faso*
[3] *Central and West African Virus Epidemiology (WAVE) for Food Security Program, Pôle Scientifique et d'Innovation, Université Félix Houphouët-Boigny (UFHB), Abidjan, Côte d'Ivoire*

Cassava Mosaic Disease (CMD) caused by Begomovirus genus is one of the main threats to cassava cultivation in Africa. Timely and accurate diagnosis of plant diseases is crucial for their early and sustainable management. However, the high cost and time-consuming limit the use of conventional methods including visual inspection in fields and laboratory diagnosis for early detection of viruses infecting crops. The aim of this study was to assess the accuracy of Nuru Plant Villagesoftware, an affordable AI tool for cassava viral diseases diagnosis in field. Five cassava leaf samples with CMD typical symptoms, and three cassava leaf samples seemingly healthy were detected CMD-infected following the Nuru Plant Village diagnosis. PCR diagnosis of these cassava leaf samples following the WAVE protocol for cassava begomoviruses detection was unsuccessful. Therefore, the samples were submitted to a rolling circle amplification followed by nanopore sequencing.Taxonomic assignation revealed that, all the samples were infected by the Begomoviruses, and contained both DNA-A and DNA-B of *African Cassava Mosaic Virus*(ACMV). The whole genome of ACMV_DNA-A was recovered from two samples having CMD typical symptoms. A mixed infection was also found between DNA-A of *East African Cassava Mosaic Virus*and both ACMV_DNA-A and ACMV_DNA-B in two samples. The Nuru Plant Village software is an accurate tool for the detection of cassava virus infection.

Mots clés : Artificial Intelligence - Rolling circle amplification - Begomoviruses detection - Nanopore sequencing - Burkina Faso.

P-03

Lipid droplets : New actors of the plant virus infection

Léna Jambou[1] (lena.jambou@u-bordeaux.fr), Nathalie Arvy[2], Charlotte QUINTEAU[2], Marguerite Batsale[2], Nathan Doner[3], Luc Sofer[2], Vincent Simon[2], Robert Mullen[3], Denis Coulon[1], Claire Bréhélin[1], Sylvie German-Retana[2]

[1] *UMR5200 Laboratoire de biogenèse membranaire, Université de Bordeaux, Villenave-d'Ornon, France*
[2] *UMR1332, INRAE, Villenave-d'Ornon, France*
[3] *Department of Molecular and Cellular Biology, University of Guelph, Guelph, Canada*

Lipid droplets (LDs) are universal organelles found in most of organisms from archaea to eukaryotes. They are composed of a phospholipid monolayer embedding various proteins surrounding a neutral lipids core. They are essential for the cell lipid metabolism, in the energy storage in seed but also in vegetative tissues and in biotic and abiotic stresses. It has been shown that LDs are hijacked by some of animal positive-sense single-stranded RNA viruses for the replication. Like animal viruses, plant positive-strand RNA viruses have to reroute host proteins, intracellular membranes, and lipids to create an optimized lipid/membrane microenvironment for their efficient viral replication compartment (VRC) assembly. However, the possible involvement of LDs in plant virus infection process has not been explored. In this study, we monitored LD biogenesis upon infection by the turnip mosaic virus (TuMV, potyvirus) in *Aarabidopsis thaliana* and *Nicotiana benthamiana.* Using the confocal microscopy, we revealed that infection by TuMV induces LD biogenesis and a relocalisation of them close to VRCs. We also showed that, when ectopically expressed in *N.benthamiana* leaves the TuMV- 6K2 (the small transmembrane viral protein that induced ER-derived vesicles crucial for replication and movement) is sufficient to induce LD formation and to recruit LDs to those vesicles. Interestingly, we also showed that other potyviral species such as potato virus A and virus belonging to another genus, barley yellow dwarf virus (BYDV, luteovirus) can also induce an increase of LDs number during *N.benthamiana* infection.

Mots clés : Potyvirus - Turnip mosaïc virus - Lipid droplets - Viral replication complexes.

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P-04

Using anti-dsRNA antibody and Nanopore sequencing for characterizing mycoviruses infecting the rice blast fungus *Pyricularia oryzae*

Laurence Blondin[1, 2] (laurence.blondin@cirad.fr), Denis Filloux[1, 2], Emmanuel Fernandez[1, 2], Henri Adreit[1, 2], Didier Tharreau[1, 2], Philippe Roumagnac[1, 2]

[1] *UMR PHIM, CIRAD, Montpellier, France*
[2] *CIRAD, INRAE, Institut Agro, IRD, PHIM Plant Health Institute, Univ. Montpellier, Montpellier, France*

Over the past decade, virus metagenomics-based approaches have revolutionized the study of the viral diversity. Noteworthy, these metagenomics approaches have often required an initial step to enrich viral nucleic acids aiming at improving virus detection efficiency. Double-stranded RNA (dsRNA) profiling, one of these viral nucleic acids enrichment-based metagenomics approaches, has proven useful for diagnoses or characterization of unreported RNA viruses. Various dsRNA enrichment methods have been developed, including the oldest traditional cellulose-based dsRNA binding and newer approaches using anti-dsRNA antibodies with specific dsRNA affinity. The added-value of this last method, primarily used in plant virology, lied in its possible adaptability to an automated and standardized format.

Here, we evaluate the application of the anti-dsRNA antibody approach followed by sequence-independent RT-PCR and Nanopore sequencing to analyze the virome of four strains of the rice blast fungus *Pyricularia oryzae*. This fungus is potentially a good study model while it is a major agricultural pathogen causing significant rice yield losses globally and it is known to host multiple dsRNA, ssRNA(+), and ssRNA(-) mycoviruses, which can be found in single or multiple infections.

Overall, we show that the diversity of identified mycoviruses includes members of the families *Botourmiaviridae*, *Narnaviridae*, *Polymycoviridae*, *Mymonaviridae*, *Partitiviridae* and *Tombusviridae*. Each of the four *P. oryzae* strains was infected by one or more mycoviruses. These preliminary results highlight the need for further studies to explore mycovirus-host interactions under various environmental conditions and stresses in order to better understand the potential impact of single or multiple mycoviruses infection on the development of the rice blast disease.

Mots clés : Mycoviruses - Rice Blast Disease - Virus Metagenomics - dsRNA Enrichment - Nanopore Sequencing.

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P-05

Evaluation of cross-protection against grapevine fanleaf virus under controlled conditions

Aleksandar Blagojevic[1] (aleksandar.blagojevic@inrae.fr), Thémis Léger[1], Jean-Michel Hily[2, 3], Wassim Rhalloussi[1, 2], Pierre Mustin[1], Shahinez Garcia[1], Myriam Hagege[1], Julie Kubina[1], Gérard Demangeat[1], Claude Gertz[1], Sophie Meyer[1], Danaé Brun[1], Véronique Komar[1], Olivier Lemaire[1, 2], Anne Sicard[1], Emmanuelle Vigne[1]

[1] *SVQV UMR-A 1131, INRAE, Colmar, France*
[2] *Laboratoire Partenarial Associé Vitivirobiome, Colmar, France*
[3] *Institut Français de la Vigne et du Vin, Le Grau-du-Roi, France*

Fanleaf degeneration, a grapevine viral disease of economic importance, primarily caused by the grapevine fanleaf virus (GFLV), lacks effective control methods. One biocontrol method developed by our team is cross-protection, a method that involves primary-infecting plants with mild strains in order to protect them against infections by related aggressive strains. We have already identified, in highly diseased vineyards from French main viticultural regions, about 100 GFLV-infected vines displaying mild symptoms. The corresponding mild GFLV isolates are candidates for cross-protection that need to be tested to assess their cross-protection potential. Due to this high number of candidates, a rapid evaluation method is required to screen them before testing them in the vineyard. We are thus performing cross-protection comparative studies between *Vitis vinifera*, GFLV-natural host, and its model hosts, *Arabidopsis thaliana* and *Nicotiana benthamiana*, under controlled conditions to determine whether these herbaceous hosts could be used to screen faster the most promising cross-protecting candidates.
A first comparative study involving GFLV-cross-protecting candidates (A or B) and nematodes cohorts (1, 2, 3) carrying different GFLV challenge variants was performed on these three hosts under controlled conditions. Infection by the challenge variants was assessed using Illumina-based GFLV-amplicon sequencing.
After 4 years of challenge, variant A protects against most of the GFLV challenger variants (0% of superinfection for plants challenged with cohorts 1 and 2 and 17% for cohort 3) while variant B protects less efficiently (13 to 50% of superinfection). Control (*i.e.* non-primary infected) grapevines showed an infection rate ranging from 73 to 91%. The results on herbaceous hosts are ongoing and will ultimately be compared with those on *V. vinifera*. These preliminary results show overall that few GFLV-superinfection events occurred for some combinations of primary/challenge variants. Although promising, further experiments are required to determine whether cross-protection can be an effective biocontrol method against GFLV.

Mots clés : Cross-protection - GFLV - Grapevine - NGS.

P-06

Relative frequency dynamics and loading of beet necrotic yellow vein virus genomic RNAs during the acquisition by its vector *Polymyxa betae*

Mattia Dall'Ara[1] (mdallara@rinova.eu), Yi guo[2], David Baldo[3], David Gilmer[4], Claudio Ratti[5]

[1] *RI.NOVA, Cesena FC, Italie*
[2] *Institute for Sustainable Plant Protection, National Research Council of Italy, Torino, Italie*
[3] *DISTAL-Plant pathology, University of Bologna, Bologna, Italy*
[4] *Institut de biologie moléculaire des plantes, CNRS, Université de Strasbourg, Strasbourg, France*
[5] *DISTAL-Plant pathology, University of Bologna, Bologna, Italie*

The beet necrotic yellow vein virus (BNYVV) is a multipartite virus with the highest number (up to five) of genomic segments among RNA viruses. Classified as a soil-borne virus, it is persistently transmitted by the protozoan *Polymyxa betae*. Previous study demonstrated that the relative frequency of the BNYVV genomic RNAs was modified depending on the host plant as well as the infected organ, resulting in distinct stoichiometric ratios between the viral RNAs. In this study, we investigate whether infection by the vector *P. betae*influences the relative abundance of BNYVV RNAs within the roots of the host plant *Beta vulgaris*. Furthermore, we examine the relative frequency of BNYVV genomic segments and the viral load of BNYVV at two different stages of *P. betae*'s biological cycle: zoospore and resting spore. Our finding offers new insights into understanding the biology of this soil borne virus and its vector. Notably, the variations in the relative accumulation of BNYVV RNAs observed in zoospores and resting spores, along with a higher viral load in zoospores compared to resting spores, invite for a consideration of the virus's replicative capacity within the vector.

Mots clés : Multipartite virus - Benyvirus - BNYVV - Protozoa - Genome formula.

P-07

Exploring the diversity of mycoviruses in grapevine associated fungi

Nuria Fontdevila Pareta[1] (nuria.fontdevilapareta@agroscope.admin.ch), Mia Messaadi[1], Nicole Lecoultre[2], Katia Gindro[2], Olivier Schumpp[1]

[1] *Virology, bacteriology, and phytoplasmology group, Agroscope, Nyon, Suisse*
[2] *Mycology group, Agroscope, Nyon, Suisse*

Fungal pathogens pose a major threat to grapevine cultivation, leading to substantial economic losses, reduced yields, and consequently a decline in wine quality. Currently, the development of alternative control methods is necessary to reduce dependence on synthetic fungicides. In this regard, mycoviruses appear as a promising strategy for the biological control of fungal pathogens, as they can induce hypovirulence in targeted fungal species. This approach would ultimately result in a reduction of disease severity without any negative effects on grapevines or beneficial organisms.
For this reason, we are analyzing fungal communities to identify viruses that show the capacity for horizontal transmission between isolates collected from the same vine stock. These include viruses with RNA-based genomes, which are widespread in fungi, and DNA viruses, which are rarer but have a greater potential for horizontal transmission. Moreover, we are analyzing the dynamic fungal collection maintained at Agroscope Changins (www.mycoscope.ch) to identify novel and/or known DNA and RNA mycoviruses that can induce hypovirulence in fungi that are most problematic in grapevines. We are particularly interested in understanding the viral diversity of lesser studied emerging fungal pathogens, such as *Phyllosticta ampelicida* (Engelm.) Aa, or major endophytes, such as *Cladosporium* sp. and *Alternaria* sp., that might represent a reservoir for mycoviruses.
Fungal strains displaying viral dsRNA profiles were analyzed using targeted RT-PCR to study the distribution of known polymycoviruses, namely Cladosporium ramotenellum polymycovirus 1 (CrPMV1) and Cladosporium cladosporioides polymycovirus 2 (CcPMV2), and high-throughput sequencing (HTS) for viral discovery. CrPMV1 and CcPMV2 were selected for their transmission potential. They were undetectable in the tested fungal strains, hence pointing towards a reduced and limited prevalence within fungal communities. Further analyses by high-throughput sequencing (HTS) revealed presence of a putative new species within the proposed new *Zetapartitivirus*genus and the known virus Alternaria alternata chrysovirus 1 (AaCV1).

Mots clés : Mycovirus - DNA virus - Cladosporium - High-throughput sequencing (HTS).

P-09

Validation d'un test de séquençage à haut débit destiné au diagnostic viral sur matrices végétales

Laëtitia Porcher[1] (laetitia.porcher@anses.fr), Benoit Remenant[1], Léna Créach[1], Muriel Bahut[2], Mathieu Rolland[1]

[1] *LSV, ANSES, Angers, France*
[2] *SFR QUASAV, Université Angers, Angers, France*

Le séquençage à haut débit est devenu une méthode de choix pour détecter sans a priori et avec un spectre large l'ensemble des agents pathogènes présents dans des échantillons simples ou complexes d'origine humaine, animale et végétale. Cependant, toute technologie a ses limites. La mise en œuvre de ce type de méthode par un laboratoire de diagnostic nécessite une validation préalable afin de s'assurer que les critères de performance de la méthode répondent aux exigences liées à son activité. Cette étape est particulièrement critique dans le cadre d'un laboratoire sous assurance qualité selon la norme ISO17025. Dans cette étude, nous présentons un processus de validation des critères de performance d'un flux de travail permettant la détection des virus et viroïdes des plantes. Ce flux est composé des étapes d'extraction de l'ARN total, d'une ribodéplétion, du séquençage Illumina et de l'analyse bioinformatique. La validation de la méthode a été réalisée en ciblant deux virus, le tomato brown rugose fruit virus (génome ARN) et le tomato leaf curl New Delhi virus (génome ADN) ainsi qu'un viroïde, le pepper chat fruit viroid (génome ARN). Selon les résultats obtenus, les principaux critères de performance (sensibilité analytique, spécificité analytique, sélectivité, répétabilité, reproductibilité et robustesse) sont comparables aux résultats d'études antérieures et entièrement compatibles avec la détection de virus et de viroïdes dans des échantillons de plantes.

Mots clés : HTS - Diagnostic - Critères de performance - Illumina - Ribodéplétion.

P-10

Reciprocal influence on viral load and effects on virulence in co-infections with latent ALSV and phylogenetically distinct viruses

Guillaume Lafforgue (guillaume.lafforgue@inrae.fr)

UMR 1332 - BFP, INRAE, Villenave-d'Ornon, France

Plant virus co-infections are ubiquitous and can dramatically alter disease outcomes. Understanding the interactions between different plant viruses is crucial for managing viral diseases and ensuring global food security. This study focuses on Apple latent spherical virus (ALSV), a bipartite virus known for its broad, asymptomatic host range, and its influence on a diverse set of co-infecting viruses. Utilizing Nicotiana benthamiana as a model host, we investigate the impact of ALSV co-infection on viruses belonging to the Bromoviridae, Virgaviridae, Alphaflexiviridae, Potyviridae, Secoviridae and Caulimoviridae families.

We hypothesize that ALSV, despite its latent nature, may significantly influence the dynamics of co-infecting viruses and vice_versa. To test this, we quantified individual viral loads in both mono- and co-infection scenarios, focusing on two key aspects:

Impact on Viral Accumulation: Does the presence of ALSV enhance, reduce, or have no effect on the accumulation of other viruses?

Genomic Formula Stability in Multipartite Viruses: any of the viruses in this study, including ALSV, have multipartite genomes. Does co-infection alter the RNA ratios of these viruses, potentially impacting their replication and functionality?

Furthermore, we assessed virulence through plant growth measurements to determine the nature of the virus-virus interactions (neutral, antagonistic, synergistic). By exploring these facets of co-infection, we aim to shed light on the complex interplay between ALSV and a diverse set of plant viruses, and ultimately determine if phylogenetic relationships influence interaction outcomes.

Our results revealed a range of interactions, including neutral, neutral-antagonistic, on viral load and synergistic effects for virulence in a particular case. Interestingly, the genomic formula of some multipartite viruses may be modulated in co-infection situations. These findings highlight the complex nature of plant-virus interactions and emphasize the importance of understanding the implications of co-infections for plant health and disease management.

Mots clés : Viral load - Co-infection - Genomic formula - Virulence - Plant viruses.

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Perspectives on detecting interactions between PPV strains using different data and methods

Olivier Cotto (olivier.cotto@inrae.fr), Sylvie Dallot, Marie Brevet, Amaury Beuzelin, Dorine Billon, Gaël Thébaud

Plant Health Institute of Montpellier, INRAE, Montpellier, France

It is now well recognized that multiple strains of a single virus often circulate in host populations and that a single host can be infected by several strains, resulting in co-infections. Much research has been devoted to characterize how co-infecting strains interact to exploit host resources, particularly in experimental settings. It is yet poorly understood how experimental results can be extrapolated to understand the epidemiological dynamics of a viral disease. Here, we aimed to characterize interactions between PPV strains, causing the sharka disease on Prunus trees, using three different data sets and methods. First, we used laboratory co-infection experiments on plum seedlings to test interactions between the strains PPV-D and PPV-Rec regarding the latency period, transmission rate and within-host virus accumulation. We did not find differences in these traits between single- and co- infections but we found that the traits were more variable in co-infections. We then investigated whether we could find similar results using epidemiological surveys. We investigated statistical independence between the frequency of co-infections and the frequency of each strain in temporal data from sharka surveillance in the Grand Est region during the 2017-2022 period. For two years, we found that the frequency of co-infections exceeded the expectation under statistical independence, suggesting that synergistic interactions can sometimes occur. Lastly, we tested whether the frequency of co-infections in a sharka survey in Serbia was consistent with the predictions from an epidemiological model. We found a deficit in co-infections compared to the model prediction. We discuss the main features of the epidemiological context of each data set and the assumptions underlying each method of analysis. We advocate that the detection of interactions between viral strains may be contingent to the study and that, more generally, interactions between viral strains may be context-dependent.

P-12

Cross-protection in plant viruses: how closely related do protecting and challenging viruses need to be?

Anne Sicard (anne.sicard@inrae.fr), Meryem Alalout, Emmanuelle Vigne, Olivier Lemaire

UMR SVQV, INRAE, Colmar, France

Cross-protection is a virus-virus antagonistic interaction well-known from plant virologists as it has been used for decades to prevent viral diseases for several plant species. It consists in infecting a plant with a primary virus in order to prevent (or interfere with) its subsequent infection by a genetically-related virus. A key parameter of cross-protection, highlighted in almost all publications dealing with this phenomenon, is that it works only between genetically closely related viruses. Surprisingly, despite the importance of this feature, the degree of sequence identity required for cross-protection to occur has not been clearly determined. Moreover, we do not know whether the sequence identity required is global or partial, i.e. whether it corresponds to the whole genome or only portion(s) of it. These questions are all the more intriguing in the case of multipartite viruses which have a segmented genome, each segment being individually encapsidated and sharing limited sequence identity with each other. *Grapevine fanleaf virus* (GFLV) is a bipartite virus considered as one of the most damaging to grapevines. By using (i) a collection of natural GFLV challenging variants spanning the known GFLV genetic diversity and with decreasing sequence identity to a primary variant, and (ii) recombinant viruses, for which large or small portions of the genomes have been swapped between distinct strains, we are studying whether cross-protection is based on a global or a partial sequence identity.

Mots clés : Cross-protection - Virus-virus-host interactions.

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PROPHYLE, une plateforme expérimentale et analytique au service de l'étiologie des maladies

Jonathan Gaudin (jonathan.gaudin@inrae.fr), Catherine Wipf-Scheibel, Isabelle Bornard, Christel Leyronas, Thierry Meylheuc, Nathalie Truglio, Eric Verdin

UR 0407 Pathologie Végétale, INRAE, Montfavet, France

PROPHYLE est une plateforme expérimentale et analytique rattachée à l'unité Pathologie végétale INRAE d'Avignon qui s'intéresse aux problématiques émergentes des cultures afin de mieux caractériser les agents phytopathogènes associés. Elle regroupe trois pôles technologiques : installations expérimentales, étiologie et microscopie. D'une part, elle vient en appui aux équipes de l'unité dans le cadre de projets de recherche, et d'autre part, elle est ouverte via des prestations à l'ensemble de la communauté scientifique et aux partenaires publics et privés souhaitant bénéficier de ses technologies et de ses installations.
Le pôle étiologie de la plateforme propose des solutions face à des maladies émergentes ou mal expliquées, principalement rencontrées par les professionnels. Il met en œuvre une démarche rationnelle de diagnostic du terrain au laboratoire afin de distinguer les problématiques d'origine biotique des désordres abiotiques par l'observation des symptômes, la prise en compte du contexte cultural, la mise en œuvre d'analyses au laboratoire et l'utilisation des ressources bibliographiques disponibles. En réponse aux demandes d'expertise, le pôle étiologie propose un diagnostic de « première intention » suite à l'envoi de photos et/ou d'échantillons pouvant aboutir à des prestations ou des projets de recherche. Un large panel d'outils disponibles peut être mobilisé : observation par microscopie optique et électronique, inoculation de plantes indicatrices, isolements microbiologiques, identification sérologique (ELISA, test bandelette), identification moléculaire (PCR, séquençage). Le pôle étiologie propose également l'isolement et la mise en collection d'agents pathogènes, la fourniture d'isolats et d'inoculum, d'insectes vecteurs et de témoins positifs (extraits d'ADN ou d'ARN). Enfin, la plateforme propose des formations et une expertise via les applications ephytia, ainsi qu'un service de diagnostic à distance par envoi de photos numériques via l'application Di@gnoview.

Mots clés : Plateforme technologique - Diagnostic - Etiologie - Maladies - Emergence.

La résistance par hypersensibilité : un moyen de lutte efficace contre le virus Y de la pomme de terre ?

Laurent Glais[1, 2] (laurent.glais@inov3pt.fr), Maryse Urvoy[1, 2], Frédéric Boulard[1, 2], Charlotte Prodhomme[1, 2], Clément Mabire[3], Caroline Viguie[4], Julien Parelon[5], Sylvie Marhadour[1, 6]

[1] *FN3PT/inov3PT, Paris, France*
[2] *IGEPP, INRAE, Institut Agro, Univ Rennes, Le Rheu, France*
[3] *SIPRE, Bretteville du Grand Caux, France*
[4] *GROCEP, Laurière, France*
[5] *Bretagne Plants Innovation, Ploudaniel, France*
[6] *IGEPP, INRAE, Institut Agro, Univ. Rennes, Ploudaniel, France*

Dans un contexte réglementaire contraint pour l'utilisation des produits phytosanitaires, la résistance génétique constitue l'un des leviers pour lutter contre le virus Y (PVY) sur pomme de terre. Ce potyvirus, transmis par pucerons, est l'un des virus les plus dommageables sur cette culture, toutes filières confondues.
Sur pomme de terre, deux types de gènes de résistance monogéniques sont décrits : des gènes de résistance extrême (*R*) qui permettent une résistance à l'ensemble des souches de PVY, et des gènes d'hypersensibilité (*N*) qui apportent une résistance souche PVY spécifique. En raison de l'absence de marqueurs moléculaires fiables pour la détection de la grande majorité de ces gènes de résistance, peu de connaissances sont disponibles sur la diversité des gènes de résistance présents dans les variétés développées, ainsi que sur leur efficacité face au PVY. Les objectifs de cette étude étaient donc d'identifier et de définir le spectre de résistance de ces gènes sur une gamme de 37 génotypes. Ces variétés ont été phénotypés en serre par inoculation mécanique avec 10 isolats PVY (4 PVYNTN, 2 PVYN-W, 4 PVYO). Un suivi de la dynamique d'expression des symptômes ainsi que des tests sérologiques réalisés sur les plantes mères et sur les plantules obtenues après replantation des tubercules fils, ont été menés. Selon ces deux critères (symptômes, ELISA), et en relation avec l'isolat PVY inoculé, 6 groupes phénotypiques ont été obtenus. Au sein de 4 de ces groupes, où des gènes *N* semblent présents, une grande diversité de sensibilité au PVY est observée en fonction du génotype plante et de l'isolat viral. Nos données montrent que la résistance par hypersensibilité est très rarement associée à l'absence de migration du PVY dans la plante. Cette résistance serait isolat spécifique, et non souche spécifique, ce qui peut limiter l'intérêt du déploiement de tels gènes de résistance.

Mots clés : PVY - Résistance - Hypersensibilité - Phénotypage - Lutte.

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Bdv2 resistance gene against barley yellow dwarf disease in wheat

Léo Blondet[1] (Leo.blondet@cirad.fr), Thomas Armand[1], Marlène Souquet[1], Elodie Pichon[1], Robin Comte[2], Emmanuel Jacquot[1]

[1] *Plant Health Institute, INRAE, Montpellier, France*
[2] *Arvalis, Montardon, France*

Barley/Cereal yellow dwarf viruses (B/CYDV), responsible of one of the most important diseases on cereals, is a complex of at least ten virus species. The most widespread and abundant species of B/CYDV in France is Luteovirus pavhordei (former Barley yellow dwarf virus (BYDV-PAV), genus *Luteovirus*, family *Tombusviridae*). BYDV-PAV is mainly transmitted by the bird cherry-oat aphid *Rhopalosiphum padi* in a persistent, circulative and non-propagative manner. For the last three decades, the main method for the management of B/CYDV in cereals was based on the use of insecticide treatments (coated seeds and/or foliar sprays). However, the recent ban on neonicotinoids in the EU and the emergence of pyrethroid-resistant aphids have sped up the development of cultivars with a phenotype resistant against virus and/or vector as an alternative to insecticides. *Bdv2* is one of the four B/CYDV resistance genes that has been introduced into wheat germplasm. The first wheat cultivar carrying *Bdv2* (i.e. cv. Tweeteo [RAGT]) has recently been made available to French growers. However, it has previously been shown that the pressure induced by *Bdv2* gene could lead to the selection of more virulent isolate(s) capable of overcoming the resistance phenotype. To avoid a rapid breakdown of *Bdv2*in fields, it is important to study i) mechanisms behind the *Bdv2* resistance and ii) links between *Bdv2* and the genetic background of wheat genotypes.

Mots clés : Luteovirus pavhordei - BYDV-PAV - Aphid - Transmission - Resistance.

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Molecular diversity of tomato leaf curl New Delhi virus (ToLCNDV) in France suggests multiple introductions in the South-east and local maintenance in weeds

Atiwich Patthamapornsirikul[1] (atiwich.patthamapornsirikul@inrae.fr), Victor Golyaev[2], Hervé Vanderschuren[2], Eric Verdin[1], Cécile Desbiez[1]

[1] *Pathologie Végétale, INRAE, Montfavet, France*
[2] *Department of Biosystems, KU Leuven, Heverlee, Belgique*

Tomato leaf curl New Delhi virus (ToLCNDV) is currently emerging in cucurbit crops throughout the Mediterranean basin. Mediterranean ToLCNDV is marked by a high genetic uniformity, distinguishing it from its Asian relatives and suggesting a monophyletic origin. In France, ToLCNDV was first detected in autumn 2020 in the South-east. Although it was not observed in 2021, it has been detected again in the same areas every year since 2022, suggesting a local maintenance or (re) introductions. Complete sequencing of French ToLCNDV isolates by Sanger or Cider-Seq indicated that all isolates belonged unambiguously to the Mediterranean clade of ToLCNDV. However, sequence analysis of 2020-2023 isolates indicated the presence of two molecular groups for DNA-A and four groups for DNA-B within French populations, with A+B combinations “A1+B1” and “A2+B2” being present from 2020 to 2023, and other combinations detected since 2022. The presence of these two distinct populations since 2020 suggests that at least two introductions of ToLCNDV had taken place at that time, and that the different populations were maintained locally over the four years, including in 2021 when the virus was not detected in crops. Surveys of weeds close to infected cucurbit crops indicated that the wild perennial cucurbits bryony (Bryonia dioica) and squirting cucumber (Ecballium elaterium) could constitute reservoirs allowing the local overwintering of ToLCNDV. The high frequency of mixed infections with several molecular groups in weeds suggests that they could play an important role in virus evolution through recombination or reassortment.

Mots clés : ToLCNDV - Evolution - Phylogeny.

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Utilisation de la Technologie Oxford Nanopore pour explorer la diversité des virus du riz en Afrique de l'Ouest et en Amérique du Sud

Martine Bangratz[1] (martine.bangratz@ird.fr), Aurore Comte[1], Mariam Barro[2], Abdoul Kader Guigma[2], Lénin Paz Carrasco[3], Hamidou Tall[4], Charlotte Tollenaere[1], Nils Poulicard[1]

[1] *UMR PHIM, IRD, Montpellier, France*
[2] *Laboratoire de Phytopathologie, INERA, Bobo-Dioulasso, Burkina Faso*
[3] *INIAP, Cantón Yaguachi, Provincia del Guayas, Équateur*
[4] *ISRA, Kolda, Sénégal*

Le riz est la céréale la plus importante pour l'alimentation humaine dans le monde. Avec les modifications de pratiques culturales, l'intensification des cultures et le dérèglement climatique, le riz est de plus en plus exposé à des épidémies et à l'émergence de nouvelles maladies, particulièrement celles d'origine virale (Anderson et al., 2004).
Actuellement, 20 virus infectant le riz dans le monde sont connus avec une majorité circulant en Asie tandis que 3 seulement ont été identifiés en Afrique et 2 en Amérique (Wang et al., 2022, Fouad et al., 2024). Au cours de ces dernières années, l'utilisation de la technologie de séquençage à haut débit par Nanopore (ONT, Oxford Nanopore Technologies) s'est développée pour le diagnostic des virus des plantes (Sun et al., 2022). L'application de cette technologie (Liefting et al., 2021) nous a permis de répondre à plusieurs objectifs, dont la caractérisation de plusieurs virus (connus ou inconnus) appartenant à différentes familles virales et présentant des organisations génomiques variées.
Ainsi, nous avons levé le voile sur la diversité intra-hôte du virus de la panachure jaune du riz (rice yellow mottle virus, *Solemoviridae*) endémique en Afrique. Nous avons également obtenu les séquences des génomes complets de virus connus mais très peu étudiés malgré leurs forts impacts sur la production rizicole qu'ils soient endémiques en Amérique (rice hoja blanca virus, *Phenuiviridae*) ou présents sur les deux continents (rice stripe necrosis virus, *Benyviridae*). Enfin, nous avons identifié et caractérisé de nouveaux virus jusqu'alors inconnus sur le continent africain, appartenant aux familles des *Potyviridae* et *Rhabdoviridae*.
Les résultats de ces recherches sur les virus du riz nous permettent de développer des outils de diagnostic efficaces pour la détection et le suivi de l'évolution spatio-temporelle des maladies virales du riz à l'échelle nationale et/ou continentale dans les rizières d'Afrique et d'Amérique du Sud.

Mots clés : Virus du riz - Technologie oxford nanopore - Afrique de l'Ouest - Amérique du Sud.

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Impact of *Ryd* resistance genes against barley yellow dwarf virus and *Rhopalosiphum padi*

Elodie Pichon (elodie.pichon@inrae.fr), Léo Blondet, Marlène Souquet, Thomas Armand, Emmanuel Jacquot

UMR PHIM, INRAE, Montpellier, France

Yellow dwarf disease (YDD) is a viral disease that causes yield losses of up to 80%. In France, *Luteovirus pavhordei* (BYDV-PAV, genus *Luteovirus*, family *Tombusviridae*) is the main viral species responsible for YDD. Phloem-restricted isolates of BYDV-PAV are mainly transmitted by the aphid *Rhopalosiphum padi*in a persistent, non-propagative manner. The use of systemic insecticides (i.e. neonicotinoids, NNI) is an effective method of limiting the spread of YDD. However, since the European ban on NNI, alternative control methods such as the use of resistant/tolerant varieties need to be considered. In this study, the effect of 13 barley cultivars (i.e. carrying the *Ryd2*, *Ryd3*, *Ryd2/3*or *Ryd4* resistance genes) on barley/BYDV-PAV/R. padi interactions was characterised by studying parameters associated with plant-virus interactions (i.e. infection rate and/or virus accumulation under different inoculum pressures). The results show that the cultivars tested have different resistance phenotypes. Most of the cultivars impacted the infection rate of BYDV-PAV and/or its accumulation (i.e. partial resistance). For two *Ryd4* cultivars, no infected plants were obtained whatever the inoculation pressure used (i.e. total resistance). These data will be progressively supplemented by additional experiments aimed at characterising the effects of i) *Ryd* genes on plant-insect interactions and ii) cultivars mixtures on the epidemic development of YDD in multi-plant arenas.

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P-19

Comparison of sequence-dependent and sequence-independent approaches for detecting sugarcane viruses in a plant quarantine context

Emmanuel Fernandez (emmanuel.fernandez@cirad.fr), Denis Filloux, Philippe Roumagnac, Philippe Rott, Jean Heinrich Daugrois

UMR PHIM, CIRAD, Montpellier, France

Implementation of sensitive and accurate methods is essential for the detection of viruses in asymptomatic plants. In this study, three diagnostic approaches were compared for detecting sugarcane viruses at the CIRAD sugarcane quarantine of Montpellier (Visacane): 1/ Sequence-dependent polymerase chain reaction (PCR) and reverse transcription (RT)-PCR, 2/ Sequence-independent virion associated nucleic acid (VANA)-based metagenomics with Illumina sequencing (VANA-Illumina), and 3/ Sequence-independent VANA-based metagenomics with Oxford Nanopore MinION sequencing (VANA-MinION). Ninety-six sugarcane samples were individually tested by PCR or RT-PCR for three known sugarcane-infecting viruses: sugarcane yellow leaf virus (SCYLV), sugarcane white streak virus (SWSV), and sugarcane mild mosaic virus (SCMMV). These 96 samples were subsequently distributed into 19 pooled samples of 4-6 individuals from a same geographical location, and processed using the two VANA approaches. SCYLV was detected by VANA-Illumina and VANA-MinION in six of seven pooled samples that contained at least one RT-PCR positive sample. SWSV tested positive by the two VANA approaches in six pooled samples with 1-5 PCR positive samples. Four of six pooled samples containing SCMMV RT-PCR positive samples tested also positive by VANA-Illumina and VANA-MinION. One of these six samples was positive by VANA-Illumina only and the remaining was negative using both VANA approaches. SCMMV was also detected by VANA-Illumina in one pooled sample that was considered free of SCMMV by RT-PCR. Fifty-three (93%) of the 57 detection results (19 pooled samples x 3 viruses) were identical regardless of the diagnostic approach. VANA-Illumina and VANA-MinION approaches also resulted in the detection of additional viruses not tested by PCR or RT-PCR, including sugarcane bacilliform virus and two unknown geminiviruses that were found by VANA-Illumina and VANA-MinION in five and two pooled samples, respectively. Metagenomics-based diagnosis of sugarcane viruses appears very promising although the sensitivity of this approach remains to be improved.

Mots clés : Sugarcane - Diagnostic - PCR - HTS.

P-20

Retour sommaire

Genetic and pathogenic diversity of Rice stripe necrosis virus (RSNV) in Ecuador

Adeline Pinault[1] (adeline.pinault@ird.fr), Lenin Paz Carrasco[2], Roberto Evaristo Celi Heran[2], Anthony Roberto Valle Choez[2], Carolina Jacqueline Panchana Torres[2], Issiaka Bagayoko[1], Martine Bangratz[1], Aurore Comte[1], Emma-Louise Jaffré[1], Séverine Lacombe[1], Laurence Albar[1], Eugénie Hébrard[1], Nils Poulicard[1]

[1] *UMR PHIM-Equipe VICITA, IRD, Montpellier, France*
[2] *Estación Experimental Litoral Sur, INIAP, Cantón Yaguachi,Provincia del Guayas, Équateur*

Rice stripe necrosis virus (RSNV) is a bipartite virus composed of two positive single-stranded RNA fragments, belonging to the genus *Benyvirus*(*Benyviridae*). Identified in West Africa from the 1980's, RSNV became a serious constraint on rice production in South America since the 2000's. In Ecuador, RSNV currently cause yield losses up to 33%. This virus is transmitted to rice by a soil protist, *Polymyxa graminis* f. sp. *colombiana* (Pgcol, *Plasmodiophoridae*), an obligate endoparasite of rice plant roots. Despite the significant impact of the disease on rice production in two continents, very limited genomic and epidemiologic data are available on RSNV and its vector. In order to broaden knowledge of this pathosystem, epidemiologic surveys were conducted in different rice-producing areas of Ecuador. First, we confirmed the presence of i) RSNV on symptomatic rice leaves by serological and molecular diagnostic tools and ii) Pgcol in rice roots by molecular detection and microscopic observations. In addition to several partial sequences, we obtained three complete genomes by Sanger, Nanopore ONT and/or, smallRNA-Illumina approaches. Then, we reproduced the virus cycle under controlled conditions by germinating rice grains in infectious soil collected from the RSNV-positive fields. Finally, under both controlled and field conditions, we discovered that RSNV and its vector have a much wider host range than previously reported. Actually, RSNV can infect efficiently other cereals (wheat, barley, oats, rye) and cause severe symptoms.
Overall, our study confirmed that RSNV is widespread in Ecuador and provided the first genetic information on RSNV and Pgcol in this country. In addition, although RSNV has so far only been described in rice, our work showed that other cultivated cereals could be alternative hosts for this virus and its vector. Thus, our results are important to consider for epidemiological surveillance of cereal fields worldwide.

Mots clés : Benyviridae - Cereals - Soil-borne virus - Epidemiology - Oxford Nanopore Technology, smallRNA-Illumina sequencing.

P-21

A wheat protein interacts with the barley yellow dwarf virus readthrough domain in potential autophagic bodies

Sylvaine Boissinot[1] (sylvaine.boissinot@inrae.fr), Amandine Barra[1], Christian Faye[2], Stéphane Lafarge[2], Claire-Line Marais[1], Thierry Michon[1], Sara Shakir[1], Maud Sorel[1], Syed Zaidi[1]

[1] *UMR Biologie du Fruit et Pathologie, INRAE, Villenave-d'Ornon, France*
[2] *Centre de Recherche de Chappes, Limagrain, Chappes, France*

Barley yellow dwarf virus (BYDV, genus *Luteovirus*) is the main agent responsible for yellow dwarf disease and infects major cereal crops like wheat and barley. Since BYDV is transmitted exclusively by aphids, with the recent EU regulation prohibiting neonicotinoids-based insecticides, there is a threat of BYDV viruses re-emerging at an alarming rate. As there are no durable sources of genetic resistance, efforts have been made in recent years to identify new sources of resistance in wheat and barley.
In the lab, in the frame of a collaboration with Limagrain, we used an interactomic approach to identify host proteins involved in BYDV cycle, that could be new targets in future breeding programs. A wheat cDNA library screening with two BYDV proteins leads to the identification of BYDV-interacting wheat proteins. A more detailed study of one of these candidate genes confirmed its interaction with the BYDV readthrough domain (RTD) using two independent protein-protein interaction techniques. Interestingly, *in planta* biomolecular fluorescent complementation (BiFC) assays indicated the subcellular localization of interaction in autophagic bodies. Additional experiments have been conducted and results to-date will be presented.

Mots clés : Luteovirus - Interactomic - Autophagy - Genetic resistance - Cereals.

P-22

Impact of the wheat dwarf virus on the fitness of *Psammotettix alienus*

Marlène Souquet (marlene.souquet@inrae.fr), Thomas Armand, Elodie Pichon, Emmanuel Jacquot

PHIM, INRAE, Montpellier, France

Wheat dwarf disease (WDD) is one of the most important viral diseases on small grain cereals. WDD is caused by the wheat dwarf virus (WDV), a mastrevirus transmitted exclusively by the leafhopper *Psammotettix alienus* in a persistent, non-propagative manner. Several management methods can be used to control WDD in cereal fields. However, they are poorly efficient against high disease pressures. In this context, acquiring data on the cereal-WDV-*P. alienus* is essential i) to better understand the epidemiology of WDD and ii) to identify innovative strategies against this disease. While cereal-WDV interactions have been extensively studied since first description of this viral disease, little data is currently available on biological parameters of *P. alienus*. In this work, an exploratory experiment was carried out to monitor biological parameters of *P. alienus* (e.g. sex ratio, adult lifespan, oviposition kinetics…). Conversely to already published data, preliminary results showed that under our experimental conditions leafhoppers i) live longer than expected and ii) lay eggs during their whole lifespan but with an intensity that depends on the time after reproduction. Based on these data, additional experiments have been initiated to evaluate effects of WDV on *P. alienus* key biological parameters. These experiments, the produced results and their putative epidemiological significance will be presented and discussed.

Mots clés : Geminiviridae - Leafhopper - Viral manipulation.

P-23

Multiplex or generic detection? When false negative results open a brain-teaser to develop a diagnostic test for banana streak virus

Serkan Önder[1, 2] (onderserkan@gmail.com), Sébastien Massart[1]

[1] *Integrated and Urban Plant Pathology Laboratory, Terra-Gembloux Agro-Bio Tech, University of Liège, Gembloux, Belgique*
[2] *Department of Plant Protection, Faculty of Agriculture, Eskişehir Osmangazi University, Eskişehir, Turquie*

The Banana streak viruses (BSVs), 13 species (fourth of them are unclassified) recognized by ICTV and belonging to the *Caulimoviridae* family, *Badnavirus* genus, are the most widespread virus affecting bananas and plantains, mainly causing chlorotic streaks on leaves. Five BSV species (BSGFV, BSIMV, BSOLV, BSMYV, BSVNV) are also integrated into the nuclear genome of diploid *Musa balbisiana* and B-genome hybrids. BSV isolates exhibit a high level of diversity, showing significant variation both in their genetic makeup and in their serological properties (Iskra-Caruana et al., 2014). This diversity makes inclusive diagnosis of BSV infections challenging. We previously identified false negative results of IC-PCR tests using of high throughput sequencing technologies. These false negative results triggered the development of new primer sets for the detection of BSVs species infecting *Musa* sp.
First, we sequenced 16 new BSV genomes, complementing the 69 genomes available in the NCBI database. Genome comparison enabled the design of primers following two strategies: one pair of generic primers (GenDeg) to detect all BSV species and three species-specific primer pairs for BSOLV, BSIMV, and BSMYV detection via multiplex PCR. Evaluation of performance criteria (analytical sensitivity and inclusivity/exclusivity, repeatability and reproducibility) of the PCR tests was performed. For the analytical sensitivity study, the Gendeg primer were better for BSOLV, BSIMV, BSGFV and BSCAV whereas the limit of detection for BSMYV was lower than the multiplex PCR test. The inclusivity study showed that for BSOLV and BSIMV, Gendeg had higher inclusivity than other primer combinations, while for BSMYV, Gendeg's inclusivity was lower than multiplex PCR. Repeatability and reproducibility studies are still continuing. Consequently, a "best-of-all" PCR test does not exist and each strategy has advantages and drawback that should be taken into account when implementing the detection test.

Mots clés : Banana streak virus - Detection - Degenerate primer - IC-PCR - Validation.

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Retour sommaire

P-24

Editing a susceptibility factor to create novel resistance alleles against RYMV

Laurence Albar[1] (laurence.albar@ird.fr), Laurence Dossou[1, 2], Agnès Pinel-Galzi[1], Florence Auguy[1], Sophie Chéron[1], Eugénie Hébrard[1], Marie-Noëlle Ndjiondjop[2], Daouda Koné[3], Marcos Celli[4], Sébastien Cunnac[1]

[1] *PHIM, IRD, Montpellier, France*
[2] *Africa Rice Center, Bouaké, Côte d'Ivoire*
[3] *University Cocody, Abidjan, Côte d'Ivoire*
[4] *CONICET, Cordoba, Argentine*

Gene editing offers new opportunities to develop resistant lines by targeting susceptibility factors involved in plant-pathogen interactions. The nucleoporine OsCPR5.1 and the translation initiation factor eIFiso4G.1 are two susceptibility factors required by rice yellow mottle virus (RYMV), one of the most devastating rice pathogens in Africa, to complete its cycle in the host plant. We have previously reported the development of resistant lines edited in OsCPR5.1 (Arra et al, 2023). Here, we will show how the editing of OseIFiso4G1 can also contribute to the development of sustainable resistance to RYMV.
CRISPR-Cas9 technology was used to generate lines with knock-out (KO) mutations in the susceptibility factor eIFiso4G.1 and lines with KO mutations in its paralog in the rice genome, eIFiso4G.2. As the central region of eIFiso4G.1 is involved in the interaction with the viral VPg, we also generated lines with short deletions in this domain. The different lines were evaluated for resistance to different isolates of RYMV, for resistance durability and for plant growth under controlled conditions. We showed that KO mutations in eIFiso4G.2 do not affect susceptibility, whereas KO mutations and some (but not all) deletions in eIFiso4G.1 confer high resistance. Interestingly, our results suggest that, despite a small effect on plant growth, KO mutations in eIFiso4G.1, may drastically reduce the risk of resistance-breakdown compared to known resistance alleles found in natural rice diversity. The 3D structures of edited eIFiso4G.1 with deletions were modeled and compared to natural resistance alleles using AlphaFold to better understand the interaction with VPg and predict resistance efficiency. Overall our results confirm that CRISPR-Cas9 is a promising strategy for generating RYMV resistance in elite rice varieties.

Mots clés : Resistance - Gene editing - Translation initiation factor - Rice.

Références :

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Suivre en temps réel le couvert de la pomme de terre en vue d'une protection raisonnée contre le virus Y

Frédéric Boulard[1, 2] (frederic.boulard@inov3pt.fr), Melen Leclerc[2], Stéphane Jumel[2], Christophe Langrume[2], Philippe Laty[3], David Dupré[4], Sébastien Vast[4], Philippe Dolo[5], Laurent Glais[1, 2]

[1] *FN3PT/inov3PT, Paris, France*
[2] *IGEPP, INRAE, Institut Agro, Univ Rennes, Le Rheu, France*
[3] *Comité Centre et Sud, Orléans, France*
[4] *SIPRE, Bretteville, France*
[5] *Bretagne Plants, Hanvec, France*

Le virus Y (PVY) est l'un des principaux problèmes sanitaires pour la production de plants de pomme de terre. Actuellement, la protection des plantes repose uniquement sur l'application d'huile minérale qui limite la transmission du virus par les pucerons vecteurs. Ces pulvérisations sont effectuées selon un cadencement réfléchit à l'échelle de la culture de pomme de terre. Cependant, afin d'optimiser ces applications, il devient essentiel de réfléchir leur fréquence à l'échelle de la variété, en considérant leur dynamique de croissance foliaire.
Afin de suivre localement la dynamique du couvert végétal en temps réel, quatre sites expérimentaux ont été équipés de capteurs fixes à transmission sans fils permettant d'acquérir des données temporelles avec une forte résolution. En plus de données météo classiques nous avons déployé des capteurs à ultrason permettant de suivre la hauteur du couvert ainsi que des capteurs de réflectance (rouge et proche -infra-rouge) utilisés pour obtenir des indices de végétations tels que le NDVI ou le EVI2.
La confrontation des données collectées automatiquement par les capteurs à celles acquises manuellement par des mesures destructives ou non, a montré l'efficacité des capteurs à rendre compte du développement local des plantes. Les résultats ont révélé une forte variabilité génotypique, inter-annuelle et géographique, dans le développement des couverts, soulignant l'importance d'un suivi localisé et en temps réel. A terme, l'enjeu est d'intégrer en direct les dynamiques de croissance des plantes dans les recommandations techniques, afin d'adapter précisément la stratégie de pulvérisation des huiles minérales. Après validation de la méthode, cette approche pourra être généralisée à tous les couverts et permettra d'optimiser la protection des plantes contre le PVY tout en contribuant à une gestion plus durable des cultures.

Mots clés : PVY - Lutte - Capteurs - Couvert végétal.

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Identification of sugar beet cultivars that exhibit antixenosis using high-throughput video-phenotyping of insect vector behaviour.

Gabin Mardoc[1] (gabin.mardoc@inrae.fr), Véronique Brault[1], Yuan Gao[2], Silène Lartigue[2], Vincent Calcagno[2], Quentin Chesnais[1]

[1] *UMR SVQV, INRAE, Université de Strasbourg, Colmar, France*
[2] *Institut Sophia Agrobiotech, Université Côte d'Azur, INRAE, CNRS, Sophia Antipolis, France*

Phytoviruses, which are mainly transmitted by insect vectors, have major impacts on agricultural yields. The recent ban of neonicotinoids has led to the search for alternatives and the launch of breeding research programs, looking for resistance traits in plants that are likely to disrupt the feeding behavior of vectors (antixenosis) and, consequently, limit viral transmission. The ANR Ecophyto-Maturation 'AGIR' project is pursuing this approach, as its main objective is to identify a protection strategy to sustainably manage sugar beet yellows (caused by four different viruses, all transmitted by aphid vectors) and limit yield losses. In this work, our initial objective is to develop and evaluate the effectiveness of a high-throughput video-phenotyping method for discriminating and selecting sugar beet cultivars of interest on the basis of their abilities to alter aphid feeding behavior. So far, this tool has been used for an initial screening phase (on 96 sugar beet cultivars) and has produced promising results, leading to an initial pre-selection of interesting cultivars. For example, we have been able to show that aphid immobility time was up to 2.75 times superior on certain sugar beet cultivars than on others, which suggests that the feeding behaviour of these aphids is also facilitated to the same extent. Subsequently, we will use the electropenetrography (EPG) technique to assess whether the results obtained by the video-phenotyping tool are confirmed and to investigate further the underlying mechanisms of behavioural alterations. Finally, viral transmission tests will be carried out to confirm that altered feeding behavior can limit the transmission of the viruses responsible for sugar beet yellowing.

Mots clés : Aphids - Video-phenotyping - Antixenosis - Plant-insect interactions - Phytoviruses.

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EUPHRESCO III : De la coordination européenne à la coordination mondiale, renforcer la programmation et la collaboration en matière de recherche phytosanitaire

Pascal Gentit[1] (pascal.gentit@anses.fr), Jasmine Zorilla[2], Géraldine Anthoine[3], Aude Chabirand[4], Philippe Reignault[3], Baldissera Giovani[2]

[1] *Laboratoire de la Santé des végétaux - Unité BVO, ANSES, Angers, France*
[2] *Euphresco, EPPO, Paris, France*
[3] *Laboratoire de la Santé des végétaux, ANSES, Angers, France*
[4] *Laboratoire de la Santé des végétaux - Unité RAPT, ANSES, Saint Pierre, La Réunion*

L'introduction d'organismes nuisibles s'est accrue ces dernières années en raison de l'augmentation des échanges mondiaux et du changement climatique. Néanmoins, les ressources et budgets alloués à la recherche phytosanitaire ont globalement diminué. La mutualisation des ressources pour répondre aux défis communs peut répondre à certaines des difficultés rencontrées par les chercheurs et leurs bailleurs de fonds.
Le réseau Euphresco a été développé pour jouer un rôle de plateforme de coordination internationale de la recherche phytosanitaire et de son financement, dans le but de réduire la fragmentation et la duplication des activités de recherche nationales et internationales. Euphresco ayant été largement considéré comme une structure de coordination européenne, la nécessité d'un réseau mondial de coordination de la recherche a été identifiée par les autorités phytosanitaires de nombreux pays.
En s'appuyant sur les bases développées par le réseau Euphresco et en intégrant les réseaux existants dans une structure commune, le projet EUPHRESCO III vise à renforcer la coordination de la recherche phytosanitaire et à jeter les bases d'une coordination mondiale de la recherche phytosanitaire.
Le réseau EUPHRESCO III s'appuie sur les activités d'organisations qui coordonnent depuis longtemps la santé des plantes dans leur région : ACIAR, APAARI, CABI, CFIA, CIHEAM-Bari, EUPHRESCO, INIA-CSIC, KHA, NIBIO, NVWA, PBRI, PFR, USDA.
Dans cet objectif, le projet Euphresco III a reçu un financement du programme Horizon Europe « Widening participation and strengthening the European Research Area » afin d'améliorer la coordination des programmes nationaux, régionaux et mondiaux de financement en recherche et innovation.

Mots clés : Recherche - Financement - Coordination - Phytosanitaire.

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P-29

Exploring the role of dodder as a potential plant virus vector in natural environments

Charlotte Julian[1, 2] (charlotte.julian@cirad.fr), Virginie Ravigné[1, 2], Hugo Fontes[3], Laurence Blondin[1, 2], Emmanuel Fernandez[2, 4], Denis Filloux[2, 4], Philippe Roumagnac[2, 4]

[1] *UMR PHIM, CIRAD, Montpellier, France*
[2] *PHIM Plant Health Institute, Univ. Montpellier, CIRAD, INRAE, Institut Agro, IRD, Montpellier, France*
[3] *Institut de recherche pour la conservation des zones humides méditerranéennes, Tour du Valat, Le Sambuc, Arles, France*
[4] *PHIM Plant Health Institute, Univ. Montpellier, CIRAD, INRAE, Institut Agro, IRD, CIRAD, UMR PHIM, Montpellier, France*

The role of insects as plant virus vectors is well established and extensively studied, both in the field and in the laboratory, to enhance our understanding of plant virus transmission. In contrast, the role of parasitic plants such as dodder (*Cuscuta* spp.) as plant virus vectors in natural environments remains largely unexplored even though numerous studies have shown that several plant viruses can be transmitted from an infected to a healthy plant through a bridging dodder. This potential for viral transmission by parasitic plants could provide valuable insights for viral epidemiology studies.
In this study, we focused on dodder, an annual epiphytic plant characterized by fine, thread-like stems that wrap around host plants to parasitize them. Our overall objective was to analyze the viral communities present in dodder stems and compare them to those of nearby parasitized and non- parasitized plants. We collected in a grazed meadow adjacent to the Tour du Valat in Camargue samples of dodder and three surrounding species, both parasitized and non-parasitized, including *Lotus tenuis* (Fabaceae), *Convolvulus arvensis*(Convolvulaceae), and *Festuca arundinacea* (Poaceae). All samples were individually processed using the virion associated nucleic acid (VANA)-based metagenomics approach. The preliminary results indicate that plant viruses belonging to several plant virus families (e.g. *Betaflexiviridae*, *Closteroviridae*, *Rhabdoviridae*, etc.) are shared by dodder plants and neighboring parasitized and non- parasitized plant species.

Mots clés : Dodder (Cuscuta spp.) - Parasitic plants - Viral transmission - Metagenomics - Viral communities.

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P-30

Unraveling structural processes of phyllosphere viral communities in grasslands under severe environmental constraints

Mathis Gheno[1, 2] (mathis.gheno@cirad.fr), Oumaima Moubset[1, 2], Hugo Fontes[3, 4], Emmanuel Fernandez[1, 2], Charlotte Julian[1, 2], Laurence Blondin[1, 2], Serge Galzi[1, 2], François Massol[5, 6], François Mesleard[3, 4], Matthieu Barbier[1, 2], Philippe Roumagnac[1, 2], Virginie Ravigné[1, 2]

[1] *UMR PHIM, CIRAD, Montpellier, France*
[2] *PHIM Plant Health Institute, Univ Montpellier, CIRAD, INRAE, Institut Agro, IRD, Montpellier, France*
[3] *Tour du Valat, Le Sambuc-Arles, France*
[4] *UMR CNRS-IRD, IMBE, Avignon Université, Aix-Marseille Université, IUT d'Avignon, Avignon, France*
[5] *UMR 8198 Evo-Eco-Paleo, SPICI Group, University of Lille, Lille, France*
[6] *U1019-UMR 8204-CIIL-Center for Infection and Immunity of Lille, CNRS, CHU Lille, Institut Pasteur de Lille, Lille, France*

Plant virus community ecology is still poorly understood. Specifically, the biotic and abiotic factors that shape viral communities in natural settings remain little studied. However, the swift advancement of sequencing technologies and metagenomics techniques has revolutionized our ability to detect and inventory plant viruses in both natural and agriculture-impacted ecosystems. Here, we have leveraged the latest viral metagenomics techniques to investigate the different factors structuring viral composition in Camargue grasslands, which are subjected to intense environmental constraints such as salinity, climate variability, and limited water access. Based on 42 sampling sites with nine replicates each, we have identified 251 viruses in 239 plant species. These inventories further enabled us to build community matrices describing plant and viral species in sites, as well as metadata describing the environment and plant characteristics. The complexity of the available data, in terms of uneven dimensionalities (few samples compared number of species/variables), variable types (binary, continuous, percentage) and variable diversity (e.g. soil physico-chemical characteristic and land-use) poses challenges for statistical analysis and interpretation. To address this, we categorized all available explanatory variables by the type of process type that might approximate, either environmental filtering or dispersal. We further clustered similar samples inside those categories and used a community detection model applied to networks: namely the Stochastic Block Model (SBM). Congruence analyses on the clusters selected by the SBM were conducted to link community structures to process approximation structure. These analyses were complemented with a selection of generalized linear models explaining viral richness. We show that virus composition is only explained at 19 % by environmental filtering and dispersal, suggesting that viral composition is probably mostly structured by ecological drift or unmeasured variables. On the other side viral richness was explained at 50% by plant cover heterogeneity, plant biomass and surrounding land-uses.

Evolutionary Drivers of Plant Virus Replication: Deep-Learning and AlphaFold2-Predicted Features of RdRp Folding combined with Flexibility-mediated Function Enable RNA Virus Characterization

Rachid Tahzima[1] (rachid.tahzima@uliege.be), Justine Charon[2], Thierry Michon[2], Sébastien Massart[1]

[1] *Laboratory of Plant Pathology, TERRA, Gembloux Agro-BioTech, University of Liège - Gembloux Agro-BioTech, Gembloux, Belgium*
[2] *Fruit Biology and Pathology Unit, National Research Institute for Agriculture, Food and Environment (INRAE)/University, Bordeaux, France*

The RNA-dependent RNA polymerase (RdRp) functions in RNA plant viruses are demonstrably modulated by native substates of dynamic and interconvertible structural features. Many of these are populated by essential flexible or intrinsically disordered regions (IDRs) that lack stable 3D structure and constitute nearly 16% of conserved RdRp domains, across the major *Riboviria* lineages. Conversely, most structural models of RdRps are typically agnostic of multiple conformations and their fluctuations, whether derived from protein structure predictors or from experimentally resolved crystal structures, as they do not consider dynamic conformer sets.
In this work, we highlight how sequence-based physicochemical drivers of protein flexibility within the conserved RdRp motifs, combined with advanced deep-learning algorithms such as AlphaFold2 (AF2), can help efficiently infer RdRp structural variability. Based on a large and representative dataset of RdRp proteins sequences from diverse publicly available repositories (n=500.000), we used AF2 protein structure confidence prediction scores, which largely capture IDRs, in combination with state-of-the-art evolutionary-driven flexibility determinants, to dissect RdRp structural features. Through key examples, our approach illustrates how unique structure-encoded preferences in sequence-function relationships can help estimate the global RdRp structural diversity as well as accelerate plant RNA virus characterization through quantitative, robust, and accurate annotations of novel or divergent RdRps mapped using Uniform Manifold Approximation and Projection (UMAP).
Finally, our coarse-grained structure/IDR-based functional depiction of RdRp proteins offers concrete perspectives on an integrative framework to better understand viral replication in early disease stages and protein-protein affinities through the context-sensitive folding behavior of these hallmark viral proteins. Beyond the characterization of novel RNA viruses, this approach has the potential to impact our understanding of the emergent global plant virosphere and to redefine the boundaries of its evolutionary landscape.

Mots clés : Evolution - AlphaFOLD2 & Structural Virology - Ai-based Plant Virus Characterization - RdRp (RNA-dependant RNA polymerase) - Intrinsic Disorder.

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Assessment of the role of mirabelle plum in sharka epidemics in northeastern France

Marie Brevet[1] (marie.brevet@inrae.fr), Guillaume Roch[2, 3], Margaux Champagne[4], Marion Delame[5], Quentin Hoffmann[6], Gaël Thébaud[1], Sylvie Dallot[1]

[1] *UMR PHIM, INRAE, Montpellier, France*
[2] *CEP innovation, Lyon, France*
[3] *GAFL, INRAE, Montfavet, France*
[4] *FREDON Grand Est, Malzeville, France*
[5] *SRAL, DRAAF Grand Est, Strasbourg, France*
[6] *AREFE, Vigneulles-lès-Hattonchâtel, France*

The Grand Est region is the leading region for the production of damson (*Prunus domestica* subsp. *insititia*) and mirabelle (*Prunus domestica* subsp. *syriaca*) plums. Damson production is threatened by sharka, caused by the plum pox virus (PPV), which is spreading in this region and controlled by visual inspection of the orchards and removal of symptomatic trees. In contrast, only very few symptoms have been reported on mirabelle leaves and fruits. Therefore, sharka is not surveyed on this species, despite the lack of scientific evidence on its susceptibility to the main PPV strains and on its potential role in sharka epidemics.
We combined orchard surveys with experimental assays under controlled conditions (i) to estimate the prevalence of PPV in mirabelle orchards and (ii) to evaluate the susceptibility of two mirabelle cultivars (P1725 and P1510) to PPV-M, PPV-D and PPV-Rec isolates after graft- and aphid-inoculation in comparison with a susceptible damson cultivar (P3066).
The presence of PPV was assessed by DAS-ELISA in 7102 leaf samples collected from 4481 trees in 53 mirabelle orchards. PPV was detected in 15% of the orchards, but the overall prevalence was very low (1.7%). The virus was detected mainly in rootstock suckers (2.6% among tested suckers) and occasionally in mirabelle leaves (0.25% of all mirabelle samples tested), on which no symptoms of sharka were observed.
Experiments showed that both of the tested mirabelle cultivars were susceptible to the three PPV strains regardless of the inoculation method, with typical PPV symptoms on the leaves. Key parameters of the infection cycle (transmission rate to test plants, incubation period, viral load, and retransmission rates by aphids from PPV-infected plants) showed that the susceptibility of the mirabelle cultivars is lower than that of the damson cultivar and depends on the PPV strain. Apparent discrepancies between survey and experimental results are discussed.

Mots clés : Plum pox virus - Disease control - Epidemiology.

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Aetiology and control of mealybug wilt disease of pineapple in Reunion Island

Delphine Massé[1, 2] (delphine.masse@anses.fr), Nathalie Cassam[1], Bruno Hostachy[1], Pierre-Yves Teycheney[3], Pierre Lefeuvre[4], Jean-Michel Lett[3], Frédéric Chiroleu[3]

[1] *Anses - LSV, St Pierre, La Réunion, France*
[2] *Université de La Réunion, St Pierre, La Réunion, France*
[3] *CIRAD, UMR PVBMT, St Pierre, La Réunion, France*
[4] *CIRAD, UMR PVBMT, Department of Plant Protection, College of Agriculture, Can Tho city, Vietnam*

Pineapple production is hampered by mealybug wilt disease of pineapple (MWP) worldwide. The aetiology of MWP is thought to involve mealybugs, several species of pineapple mealybug wilt-associated virus (PMWaV, genus *Ampelovirus*) and potentially other viruses of genera *Badnavirus*, *Secovirus* and *Vitivirus*but is not entirely elucidated yet. We addressed this issue through a study of the distribution and prevalence of five viruses previously described as associated with MWP, in 15 plots of cultivar 'Queen Victoria' cultivated across Reunion Island, and a statistical analysis of the association of some of these viruses with MWP symptoms.
We collected 450 symptomatic and asymptomatic leaf samples and indexed them by PCR analysis for the presence of three ampeloviruses (PMWaV1, PMWaV2 and PMWaV3) and two badnaviruses (PBERV and PBCOV). We found that 93% of the analyzed samples were infected with at least one of these viruses and 76% were co-infected by two to four viruses. The most prevalent viruses were PMWaV1 (78%) and PBCOV (87%), whereas PBERV was not detected. The presence of viruses was significantly associated with symptoms of leaf dieback, wilting and curling, but not with leaf reddening or yellowing, which are nonetheless described as typical MWP symptoms, but which also correspond to leaf reactions to other biotic and abiotic stresses. Additionally, viral infections and MWP symptoms were significantly less prevalent in plots using vitroplants (VPs) as planting material than in plots using suckers, suggesting that VPs could help limit the impact of MWP in Reunion Island.

Mots clés : Ananas comosus - Pineapple mealybug wilt disease - Aetiology - Virus - Vitroplants.

The aphid-transmission of nanoviruses involves an atypical Helper Component

Alice Mercey[1] (alice.mercey@gmail.com), Laura Di-Pietro[1], Mathilde Villegas[1], Jean-Louis Zeddam[1], Elodie Pirolles[1], François Hoh[2], Stéphane Blanc[1]

[1] *PHIM-VIROM- Équipe MULTI, CIRAD, INRAE, IRD, Sup Agro, Université de Montpellier, Montferrier-sur-Lez, France*
[2] *Centre de Biologie Structurale, INSERM, CNRS, Université de Montpellier, Montpellier, France*

The transmission of plant viruses by vectors is often facilitated by a helper component (HC). A HC is a non-structural protein encoded by the virus and produced in the infected plant. It acts as a molecular bridge between the viral particle and a receptor located within the vector. The HC allows either retention/release in the vector's mouth parts in the case of non-circulative transmission, or recognition/internalization within gut cells in the case of circulative transmission. Due to this mode of action, nearly all HC described can be acquired together with or prior to the virus particles, the opposite acquisition sequence leading to transmission failure. Thus far, the only exception to this rule is the NSP protein of the nanovirus faba bean necrotic stunt virus(FBNSV), transmitted by aphids in a circulative non-propagative manner. NSP can be acquired both before and after viral particles with similar transmission efficiency, questioning its mode of action. As opposed to all other characterized HCs, no interaction between NSP and the FBNSV capsid protein (CP) has been demonstrated. In contrast, NSP has been shown to interact with the replication protein M-Rep and, surprisingly, we could detect M-rep in the vector's midgut cells, conditional on the presence of NSP. To elucidate the NSP mode of action and its possible viral partners in the transmission process, we modeled the structures of CP, NSP and M-Rep and predicted the putative interaction sites between CP-NSP and M-Rep-NSP using Alphafold 3. These predictions will guide site-directed mutagenesis targeting residues of M-Rep and/or CP, specifically hindering the interactions with NSP, to evaluate their impact on virus transmissibility. These experiments will provide crucial insights into the apparent atypical transmission process of nanoviruses, potentially revealing a novel mode of action for helper components.

Mots clés : Vector-transmission - Plant - Aphids - Nanovirus - Helper-components.

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Chasing phantoms

Justine Brodard[1] (justine.brodard@agroscope.admin.ch), Beatrix Buchmann[2], Markus Bünter[2], Nathalie Dubuis[1], Marc Passerat[1], Olivier Schumpp[1], Arnaud G. Blouin[1]

[1] *Agroscope, Nyon, Suisse*
[2] *Agroscope, Wädenswil, Suisse*

Safe exchange of plant material relies on accurate diagnostic tools and up-to-date lists of regulated pathogens. However, some of these lists include obscure diseases, often reported only once in non-peer-reviewed publications such as conference proceedings. In a global effort to remove such agents from all regulatory lists, a recent publication identified over 120 agents/diseases for which essential information was lacking (Tzanetakis et al. 2024). These included agents associated with symptomatic plants, presumed to be of viral nature but for which no plant material was available and for which no sequence information was accessible. They were designated as phantoms. The initial list of these agents included the cherry rosette virus (CRV), which was first described as a nepovirus in cherry trees in Switzerland in 1994. In their study, Brown *et al.* described a novel nematode, *Longidorus arthensis,* as the vector of CRV. However, no isolate or sequences of this virus was available until today. Following an analysis of the collection held at Agroscope, a CRV isolate, designated #858, was identified. Isolated in 1997 on cherry trees in central Switzerland, it was then successfully inoculated on various herbaceous plants in the greenhouse. Infected *Nicotiana clevelandii* stored in the freezer for more than 20 years was successfully re-inoculated in 2024. The genome obtained confirmed that CRV is a nepovirus, and clusters with grapevine Tunisian ringspot virus (only RNA2 available), Paris mosaic virus 1 (Actinidia nepovirus A), Sichuan green pepper virus and Nitraria roborowskii nepovirus. Full characterisation of the viruses is underway and key results will be presented. With isolates and sequences now available, this virus has been removed from the list of phantom agents.

Mots clés : Cherry rosette virus - Nepovirus - Prunus avium - Phantom agent - Historical collection.

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Economic inefficiencies in private management of epidemics spreading between farms

Gaël Thébaud[1] (gael.thebaud@inrae.fr), César Martinez[2, 3], Mabel Tidball[2], Pierre Courtois[2]

[1] *PHIM, INRAE, Montpellier, France*
[2] *CEE-M, INRAE, Montpellier, France*
[3] *Ecodéveloppement, INRAE, Avignon, France*

Most plant disease epidemics spread both within and between farms. However, in the absence of collective action, each farmer generally takes disease control decisions based on personal costs and benefits. It is important to identify under which conditions the combination of such private control decisions can have synergistic or antagonistic effects, and can lead to collective economic inefficiencies. We used the game theory framework to investigate these questions, considering a simplified two-period game where two farmers decide whether or not to control an epidemic on their farm. Taking the example of sharka epidemics, caused by plum pox virus in *Prunus* orchards, we characterized the game and its outcomes according to initial epidemic conditions and focused on those likely to produce economic inefficiencies. Our results show that depending on the initial infection levels, a broad range of games may arise, some of which involving synergistic or antagonistic control decisions. This means that the nature of strategic interactions between famers may change depending on the state of the epidemic. After a thorough characterization of the epidemic conditions for which private management produces collective economic inefficiencies, we investigated the expected effect of different public policy incentives aiming to reduce such inefficiencies.

Mots clés : Epidemiology - Economics - Strategy - Collective action - Plum pox virus.

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Isolement, caractérisation et maintien d'une collection unique de virus de phytoplancton à la *Roscoff Culture Collection*

Pauline Nogaret[1] (pnogaret@sb-roscoff.fr), Nathalie Simon[1], Fabienne Rigaut-Jalabert[2], Florence Le Gall[1], Estelle Bigeard[1], Michele Grego[2], Martin Gachenot[2], Priscilla Gourvil[2], Daniel Vaulot[1], Ian Probert[2], Anne-Claire Baudoux[1]

[1] *UMR Adaptation et Diversité en Milieu Marin, Station Biologique de Roscoff, Roscoff, France*
[2] *FR2424, Station Biologique de Roscoff, Roscoff, France*

Ces dernières décennies, les virus sont apparus comme une composante majeure de la biosphère marine. On compte aujourd'hui 1 à 100 milliards de virus dans chaque litre d'eau de mer. L'essor des techniques de séquençage haut-débit a mis en lumière l'immense diversité génétique des virus marins avec plus de 200,000 espèces virales recensées dans l'océan global. Malgré ces chiffres démesurés, nos connaissances sur les virus marins restent éparses et souvent conceptuelles. La vaste majorité des gènes viraux (90%) dans l'océan reste notamment non assignée par manque de séquence de référence. Nos recherches contribuent à caractériser cette composante énigmatique de la biosphère *via* l'isolement et la description fonctionnelle, génétique et structurale de nouveaux virus marins. La création d'une collection unique de virus marins au sein de la Roscoff Culture Collection (https://roscoff-culture-collection.org/) rend ces isolats disponibles à la communauté et au grand public. Cette présentation sous forme de poster présentera la diversité de virus mis à disposition à la Roscoff Culture Collection. Nous détaillerons les méthodes d'isolement, caractérisation, et conservation à long terme de ces cultures ainsi que les avantages qu'une telle collection apporte à la communauté scientifique.

Mots clés : Virus - Phytoplancton - Marin - Collection.

Strategies for developing new genetic resistances to viruses based on the susceptibility factors phosphoglycerate kinases

Geoffroy Milkoff[1, 2] (geoffroy.milkoff@inrae.fr), Lucas Laflaquière[1], Nathalie Giovinazzo[1], Mamoudou Diop[1], Zoé Terret-Welter[3], Céline Labourey[2], Jean-Luc Gallois[1]

[1] *GAFL, Inrae, Avignon, France*
[2] *Syngenta, Sarrians, France*
[3] *HM Clause, Saint-Rémy-de-Provence, France*

Plant genes encoding susceptibility factors are good targets to develop genetic resistances to viruses. Although resistances to potyviruses mainly rely on translation initiation factors such as *eIF4E*, it is important to characterize new host susceptibility factors. In this light, we investigate the role of the metabolic enzymes Phosphoglycerate kinases, which have been shown to act as susceptibility factors to several potyviruses.
We show that inactivation of *cPGK2*, that has been characterized as a natural resistance allele in the Arabidopsis accession Cvi, is not sufficient to trigger resistance to watermelon mosaic virus. We reasoned that this could be caused by the existence of a gene closely homolog to *cPGK2* - *cPGK1*- that could also be targeted by the virus. Interestingly, cPGK1 accumulates predominantly over cPGK2 in leaf cells, suggesting that knocking out *cPGK2*should not be sufficient to lower the level of PGK susceptibility factors. Moreover, significantly lower levels of *cPGK1*mRNA accumulates in the Cvi accession: the natural resistance in Cvi could simultaneously result from a mutated *cPGK2*and a lower abundance of cPGK1. We posit that Arabidopsis is a good model to understand the complexity of cPGK-based susceptibility/resistance, with the aim of developing effective resistances without altering plant development Based on this hypothesis, I will try to mimick the Cvi natural resistance to potyviruses in a susceptible *Arabidopsis thaliana* accession, by a combination of endogenous *PGK*inactivation and genetic complementation. In parallel, I will investigate the mechanisms by which the plant PGKs are recruited by the virus. Finally, I will present strategies to transfer in Tomato PGK-based resistance to poty- and potexviruses using genome editing techniques. Overall, this work should provide guidelines for designing resistance based on S genes families in crops.

Mots clés : Phosphoglycerate kinase - Susceptibility genes - Potyvirus/Potexvirus - Arabidopsis thaliana.

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Bioinformatics solutions for addressing post-processing challenges in Nanopore sequencing data

Denis Filloux[1, 2] (denis.filloux@cirad.fr), Emmanuel Fernandez[1, 2], Frédéric Mahé[1, 2], Philippe Roumagnac[1, 2]

[1] *CIRAD, UMR PHIM, Montpellier, France*
[2] *PHIM Plant Health Institute, Univ Montpellier, CIRAD, INRAE, Institut Agro, IRD, Montpellier, France*

Nanopore sequencing (Oxford Nanopore Technology) has rapidly become more accessible and widely adopted in research laboratories, offering a real-time, cost-effective, and portable sequencing technology. This advancement has significantly accelerated scientific discoveries, particularly in metagenomic studies, by enabling comprehensive analyses of diverse microbial and viral communities.
However, the inherent characteristics of Nanopore sequencing introduce several challenges. The high error rate, for instance, complicates the assembly of reads, as well as the detection and removal of Nanopore adapters and barcodes. Additionally, the ligation of adapters to DNA fragments (using the Ligation Sequencing Kit) and/or the overloading of DNA in the flowcell, appear to contribute to a substantial number of chimeric and inverted repeated reads, especially when dealing with short DNA fragments. These issues significantly complicate the processing of sequencing data from multiplexed DNA samples, as many reads are either misallocated to incorrect samples (e.g., chimeric reads) or poorly assembled (e.g., inverted repeated reads).
Existing bioinformatics tools, such as Dorado, Guppy, and Porechop, do not fully address these challenges. Consequently, we identified the need for specialized, custom-built tools designed to tackle these specific problems. To address this, we are currently developing three computational tools:

. **DeSIR**: a tool for detecting and splitting inverted repeat reads,
. **Tripatouille**: a tool for identifying and removing adapters and barcodes (custom or Nanopore), particularly suited for demultiplexing and cleaning multiplexed amplicon reads, such as those derived from the virion-associated nucleic acid (VANA)-based metagenomics approach,
. **ChimeraKiller**: a tool for detecting and splitting chimeric reads (currently under development).

These tools, still in the prototype phase, require further refinement, particularly in terms of execution speed and adjustments to sensitivity and specificity.

Mots clés : Nanopore sequencing - Bioinformatic tool - Sequencing error rate - Chimeric read - Inverted repeat.

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Understanding the role of lipid transfer proteins in luteovirus infection

Sara Shakir[1] (sara.shakir@inrae.fr), Yassine Benhamou[1, 2], Amandine Barra[1], Christian Faye[3], Claire-Line Marais[1], Christophe Sallaud[3], Maud Sorel[1], Stéphane Lafarge[3], Sylvaine Boissinot[1], Thierry Michon[1], Syed S Zaidi[1]

[1] *UMR 1332/BFP/Virology, INRAE, Villenave-d'Ornon, France*
[2] *University of Strasbourg, Strasbourg, France*
[3] *Centre de Recherche de Chappes, Limagrain, Chappes, France*

Yellow dwarf disease is an emerging threat to global cereal production [1]. It is caused by co-infection of luteoviruses and poleroviruses, which belong to two distinct families but share high identity in their 3' end of the genome known as the 'luteo-polero block'. The readthrough domain (RTD) in the luteo-polero block is critical for virus infection, yet its role in virus infection cycle remains mostly unknown [2]. In this study, conducted in collaboration with Limagrain, we established the interactome of the RTD from barley yellow dwarf luteovirus (BYDV) in *Nicotiana benthamiana* using TurboID-mediated proximity labeling to better understand its function in plant-virus interactions. Among the identified candidate proteins interacting with BYDV RTD, we found a lipid transfer protein (LTP). LTPs are known for transferring lipids from the endoplasmic reticulum to other cellular membranes and play key roles in lipid metabolism, membrane trafficking, and lipid-dependent signaling [3]. The interaction between RTD and LTPs from *N. benthamiana* and wheat orthologs was confirmed through yeast two-hybrid assays and in planta using Bimolecular Fluorescence Complementation assays. Both RTD and LTPs were found to colocalize in the cytoplasm, and the presence of RTD did not alter the localization of LTPs, suggesting a stable intracellular distribution during viral interaction. Furthermore, the interaction between these proteins appears to be cytoplasmic, consistent with the known cytoplasmic roles of LTPs. To further explore the role of the RTD-LTP interaction in the viral infection cycle, we performed virus-induced gene silencing of LTPs in *N. benthamiana*. Silencing of LTPs resulted in plant dwarfism. Virus quantification assays are currently underway to determine whether this interaction has a proviral or antiviral effect during infection.

Mots clés : Barley yellow dwarf luteovirus - RTD - Lipid transfer proteins - Interactome.

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Surveillance de *Bemisia tabaci* par high-throughput sequencing : intégration des connaissances entomologiques et virologiques pour une évaluation améliorée des risques phytosanitaires

Fauve Maertens[1] (fauve.maertens@ilvo.vlaanderen.be), Maaike Heyneman[1], Annelies Haegeman[1], Johan Witters[1], Lisa Heyman[2], Saskia Buysens[2], Kris De Jonghe[1], Wannes Dermauw[1]

[1] *Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Merelbeke, Belgique*
[2] *Viaverda vzw, Kruisem, Belgique*

La mouche blanche *Bemisia tabaci* est un ravageur mondial majeur des cultures, causant chaque année des pertes de plus d'un milliard d'euros et d'une multitude de pertes, car elle est le vecteur d'une diversité de virus phytopathogènes. Classée organisme de quarantaine dans l'UE, elle représente une menace importante lorsqu'elle est détectée sur des plantes importées de régions non européennes. Bien que présente en Belgique, sa distribution locale et les risques virologiques qu'elle faisait peser sur les cultures en serre restaient peu connus. Entre 2021 et 2024, plus de 50 populations de *B. tabaci* d'origine non européenne ont été interceptées sur des marchandises importées dans les ports d'entrée. En parallèle, des populations locales (européennes) ont été prélevées sur des cultures et plantes ornementales domestiques.
Ce travail a combiné des approches entomologiques et virologiques, en utilisant des techniques moléculaires classiques et *high-throughput sequencing* pour 1) caractériser les biotypes de *B. tabaci*, 2) analyser les communautés virales associées à ces populations et 3) détecter les mutations de résistance aux virus et aux insecticides. Grâce à cette méthodologie intégrée, la majorité des mouches blanches collectées appartenaient à deux biotypes, et une diversité de virus végétaux, incluant les crini- et bégomovirus, a été détectée. Des mutations de résistance ont également été identifiées dans les populations européennes et non européennes.
En combinant des approches d'entomologie et de virologie, cette étude a fourni une vision plus globale des risques phytosanitaires associés à *B. tabaci* et de développer une stratégie ciblée visant à protéger les cultures en serre belges des menaces virales émergentes.

Mots clés : Bemisia tabaci - High-throughput sequencing.

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Influence of viromes on the phytochemical composition of wild hop (Humulus lupulus) collected in Switzerland

Isabelle Kellenberger[1] (isabelle.kellenberger@agroscope.admin.ch), Olivier Schumpp[1], Louis Sutter[2], Sophia Corsi[2]

[1] *Agroscope, Nyon, Suisse*
[2] *Agroscope, Conthey, Suisse*

Hops (*Humulus lupulus*), a dioecious perennial climbing plant reaching up to 8 meters, is highly valued in brewing for the bitterness and aroma imparted by its female cones. High alpha acid (humulone) levels characterize bittering hop varieties, while lower concentrations define aromatic hops. In Switzerland, approximately 90% of hops are imported as pellets and there is a strong interest in developing local production using local varieties.
In 2021, 70 unique Swiss wild hop genotypes were collected and an experimental field was established. This project aims to compare local genotypes with 15 commercial hop varieties to evaluate their potential suitability for cultivation. Although hops grow rapidly and can remain productive for 20–30 years, long-term cultivation increases their susceptibility to diseases. Several of these diseases are caused by viruses or viroids which can alter the phytochemical composition of the plant (Pethybridge et al., 2002; Jelínek et al., 2012).
A detailed virome analysis of the plantation was performed to investigate viral diversity and prevalence. Double-stranded RNA extractions followed by Illumina sequencing were conducted on 79 samples, comprising 15 libraries: seven pooled samples of ten plants each, and eight individual plant samples. The virome analysis revealed the presence of several viruses and viroids commonly associated with hop cultivation, such as *Hop latent virus* (HpLV), alongside other economically significant pathogens, highlighting the diverse viral landscape in the analyzed samples.
A key aspect of this study is to explore the relationship between pathogen presence—specifically viruses and viroids—and the phytochemical composition of the hops. The findings from this research will provide valuable insights for the selection and cultivation of hop varieties, aiming to balance disease resistance and desirable brewing characteristics, while contributing to the understanding of plant-pathogen interactions in perennial crops.

Mots clés : Hop - Virome - Phytochemical.

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P-43

Assessment of the bionematicide potential of *Fabaceae* to delay the re-contamination of newly planted vines by grapevine fanleaf virus in vineyard

Lise Negrel[1, 2] (lise.negrel@inrae.fr), Sophie Meyer[1], Raymonde Baltenweck[1], Françoise Le Bohec-Dorner[1], Camille Rustenholz[1], Amandine Velt[1], Pascale Gombault[2], Philippe Hugueney[1], Olivier Lemaire[1], Gérard Demangeat[1]

[1] *UMR 1131 SVQV, Equipe Virologie et Vection, INRAE, Université de Strasbourg, Colmar, France*
[2] *Multifolia, Viâpres-le-Petit, France*

Grapevine fanleaf virus (GFLV), specifically transmitted by the soil-borne nematode vector, *Xiphinema index,* is a widely distributed *Nepovirus* responsible for fanleaf degeneration. Nematicides have been used until the 2000's to control nematode populations and protect grapevines from a rapid re-infection between uprooting and new vines plantation. Due to environmental impacts and healthy concerns, they have been banned leading the grapevine growers to a technical deadlock to control this disease. Therefore, there is a growing interest in agroecological strategies, including plant species with antagonistic effects towards *X. index*.
Here we evaluate bionematicide potentials of aerial parts and roots of four *Fabaceae*: sainfoin, birdsfoot trefoil, sweet clover, and red clover, as well as deshydrated sainfoin-pellets (VitifoliaTM). In an aqueous *in vitro* assay, either aerial or root parts all the tested plants, or both of them, exhibited bionematicide activities on *X. index*. Sainfoin pellets are effective as freshly harvested plant. Using adapted bioassays developed in greenhouse, we further demonstrate that sainfoin pellets slow down *X. index* multiplication.
Comparative metabolomic analyses of sainfoin did not reveal molecules or molecule families specifically associated to antagonistic properties toward *X. index*, suggesting that bionematicide effect is the result of a combination of different molecules rather than associated to a single compound.
Meanwhile, we have designed open-field experiments highly infected by *X. index*/GFLV pathosystem. The first open-field experiments starting 7 years ago, combining a *Fabaceae* fallow, supplemented with sainfoin VitifoliaTM pellets before plantation, shows so far a slower re-contamination of this modality compared to the controls. These preliminary results suggest that an integrated management combining complementary levers including bionematicide fallows with Fabaceae, their dehydrated compounds, innovative genetic resistances and cross-protection could eventually enable the viticulture industry to succeed in living sustainably with the fanleaf degeneration.

Mots clés : Soil-borne nematode - Xiphinema index - Fanleaf degeneration - Grapevine fanleaf vius - Bionematicide.

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Genomic and phylogenetic investigations suggest that sugarcane streak mosaic virus is vectored by at least one species of mite

Jean Heinrich Daugrois[1] (jean-heinrich.daugrois@cirad.fr), Philippe Roumagnac[1], François-Régis Goebel[2], Philippe Rott[1]

[1] *UMR PHIM, CIRAD, Montpellier, France*
[2] *UR AIDA, CIRAD, Montpellier, France*

The insect vector of sugarcane streak mosaic virus (SCSMV) is unknown. SCSMV belongs to the genus *Poacevirus* of the family *Potyviridae*. This genus currently includes four virus species: *Sugarcane streak mosaic virus, Triticum mosaic virus*, *Caladenia virus A*, and *Zoysia mosaic virus*. Triticum mosaic virus is transmitted by the wheat curl mite (WCM) *Aceria tosichella*, an eriophyid mite that failed to transmit SCSMV. This mite is also the vector of wheat streak mosaic virus (WSMV), a tritimovirus of the family *Potyviridae*. WSMV has a zinc finger like (ZFL) motif [H(X2) C X29 C(X2)C] in the HC-Pro protein that is necessary for its transmission by the WCM. However, all viruses of the *Potyviridae* family that possess a HC-Pro protein have a ZFL motif in the HC-Pro, which is therefore not specific to mite transmission. Two other well studied motifs, namely KITC and PTK, are associated with aphid-transmission of potyviruses. Among 131 species of the *Potyviridae* family, the KITC [or a similar motif that is attached to the C(X2)C motif of the ZFL motif] and the PTK motifs are present in almost all aphid-transmitted potyviruses, but are missing in all non-aphid transmitted viruses. A phylogenetic tree was constructed with the HC-Pro sequences of the 131 *Potyviridae* species and was linked to protein motifs and virus vectors. SCSMV clustered with a group of viruses spread by Aceria mites, which suggested that at least one mite species is a vector of SCSMV. Most mite species found on sugarcane belong to the Eriophyidae family with at least 10 species belonging to six genera that are distributed in Africa, America, Asia, and Oceania. Eriophyid mites are able to spread most mite-transmitted viruses. Consequently, eriophyid mites are good candidates for vectoring SCSMV and should be identified and tested in geographical locations where this virus is present.

Mots clés : SCSMV - Vector - Mite - Sugarcane.

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Caractérisation partielle du virome d'*Ipomoea batatas* (*Convolvulaceæ*) et de *Dioscorea sp.* (*Dioscoreaceæ*) dans le Grand-Ouest français

Gaël Revert[1, 2] (gael.revert.ext@anses.fr), Mathieu Rolland[1], Laëtitia Porcher[1], Michèle Visage[1], Pascal Gentit[1]

[1] *Laboratoire de la Santé des Végétaux, ANSES, Angers, France*
[2] *AgroParisTech, Palaiseau, France*

L'augmentation des échanges commerciaux de matériel biologique à l'échelle planétaire est à l'origine de l'introduction de nombreux organismes nuisibles. Ce mécanisme qui conduit à l'émergence de nouveaux agents pathogènes est d'autant plus efficace lorsque ce matériel est destiné à la production de denrées alimentaires et que son mode de reproduction est végétatif. En effet, contrairement à la multiplication par semences cette méthode de multiplication assure le maintien du virome et peut conduire à une accumulation de nombreux virus dans le temps et dans une même plante. Cette étude s'est intéressée à certaines de ces cultures dont la reproduction végétative est le mode de production de plants privilégié et dont l'implantation sur le territoire est nouvelle ou bien connait un renouveau ; parmi elles, la patate douce (*Ipomoea batatas*) et l'igname de Chine (*Dioscorea polystachya*). Les travaux menés ont visé à caractériser le virome de ces plantes, de manière non exhaustive. Pour ce faire, du matériel végétal a été récolté sur des exploitations provenant du Grand-Ouest français. Suite aux extractions d'acides nucléiques, les analyses par PCR polyvalentes qui ont été menées (*Badnavirus*, *Begomovirus*, *Cucumovirus*, et *Potyvirus*) ont permis d'identifier le *Sweet potato badnavirus B* (genre *Badnavirus*), le *Sweet potato pakakuy virus* (genre *Badnavirus*), et le *Sweet potato virus G* (genre *Potyvirus*), sur *Ipomoea batatas* et de détecter un nouveau *Badnavirus* et un nouveau *Potyvirus* sur *Dioscorea polystachya*.

Ce travail a été financé par le Ministère en charge de l'Agriculture.

Mots clés : Virus - Patate douce - Igname - PCR - Santé des végétaux.

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Virome of cross-protected grapevines under natural conditions: what else is in there?

Julie Kubina[1] (jkubina@unistra.fr), Jean-Michel Hily[2], Pierre Mustin[1], Claude Gertz[1], Gérard Demangeat[1], Anne Sicard[1], Olivier Lemaire[1], Emmanuelle Vigne[1]

[1] *UMR-A 1131-SVQV, INRAE, Colmar, France*
[2] *LPA Vitivirobiome, IFV, Colmar, France*

Fanleaf degeneration is a worldwide disease causing a severe decline of vineyards with serious economic losses. Its main causal agent is the grapevine fanleaf virus (GFLV), a virus transmitted from grapevine to grapevine by an ectoparasitic nematode. While GFLV is depicted as one of the most severe viruses of grapevines, infected-vines are not always symptomatic and show a wide range of symptoms with varying degrees of severity. The lack of effective measure to control this disease led us to explore mild strain cross-protection (MSCP) as a biocontrol method. In MSCP, primary infection with a mild isolate is used to prevent secondary infection(s) by related severe variant(s). Efficient and long-term MSCP relies on the selection of isolates causing mild symptoms on the cultivar of interest and able to prevent subsequent infection by challenger variants present in its growing environment.
In this study, the efficacy of MSCP against fanleaf degeneration was assessed by monitoring 1,950 primary-infected vines with mild GFLV isolates (originated from Burgundy region in France) that were implanted in the same viticultural region in a diseased commercial plot exhibiting severe symptoms before uprooting. After 14 years, 81% of the vines displayed mild symptoms with good fruit production aptitudes. In order to determine if superinfection occurred, the infectious status of about 200 vines showing contrasted phenotypes were determined using high-throughput sequencing technologies (total RNA and amplicon sequencing). After phylogenetic analysis, more than 70% of the GFLV sequences grouped into the same clades as those of the primary isolates, indicating low level of superinfection and stability of the mild isolates. Our results will be discussed with the aim at developing MSCP as a workable approach to prevent fanleaf degeneration while taking into account the complex virome of grapevines in vineyards.

Mots clés : Cross-protection - GFLV - Mild symptom.

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Attempts at translating resistance to potexvirus from Arabidopsis to tomato by inducing loss-of-susceptibility

Caroline Lebaron (caroline.lebaron@inrae.fr), Jean-Luc Gallois

UR1052 GAFL, INRAE, Montfavet, France

A major goal of plant breeding is to translate genetic resistances to viruses across species and in the genetic background of choice. In the case of susceptibility factors, resistance to viruses can be achieved by genome editing following the inactivation (knock-out) or modification of Susceptibility (S) genes, but how these mechanisms can be translated from model plant to crops is not always clear. Moreover, for breeding purposes, resistance should not be developed at the expense of the plant development. Here, we focus on two S genes involved in resistance to potexvirus, namely *nCBP* (*novel Cap Binding Protein*), encoding a protein related to the family of eIF4E translation initiation factors, and *EXA1* (*Essentials for poteXvirus Accumulations 1*), which encodes a GYF domain-containing protein.
It has been previously shown that i) inactivating *nCBP* or *EXA1* in Arabidopsis limit the viral propagation of the potexviruses PlAMV (Hashimoto *et al.* 2016, Plant J) and that ii) *EXA1* and *eIF4E* family members play additive roles on the accumulation of Potexvirus (Nishikawa *et al.* 2023 J Virol.). Here, to translate those results to crop, we describe the production of ncbp and exa1 mutants in different tomato genetic backgrounds, using genome editing techniques. We will present preliminary results on resistance to viruses as well as effect of the mutations on the plant and fruit development. This might provide useful insight on the transferability of those S genes for breeding purposes, as well as information on the trade-off between resistance and development.

Mots clés : Gene editing - Potexviruses - Genetic resistance.

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Exploration du lien entre virome et agressivité chez *Plasmopara viticola*, l'agent du mildiou de la vigne

Chantal Faure[1] (chantal.faure@inrae.fr), Thierry Candresse[1], Isabelle Mazet[2], François Delmotte[2], Simon Labarthe[3, 4], Corinne Vacher[2], Armelle Marais[1]

[1] *BFP, INRAE, Villenave-d'Ornon, France*
[2] *SAVE, INRAE, Villenave-d'Ornon, France*
[3] *BIOGECO, INRAE, Cestas, France*
[4] *Pléiade, INRIA, Talence, France*

Le projet VITAE, lauréat du Programme Prioritaire de Recherche « Cultiver et Produire Autrement », a pour objectif de produire de nouvelles connaissances pour favoriser la transition agroécologique en viticulture. Un des axes de recherche du projet est d'identifier des microorganismes antagonistes du mildiou de la vigne, causé par l'oomycète biotrophe obligatoire *Plasmopora viticola*. Dans ce contexte, nous nous intéressons à la composition et à l'impact potentiel du virome des souches de mildiou sur leur agressivité au travers de deux hypothèses : (i) les souches de mildiou les moins agressives hébergent un plus grand nombre de mycovirus, (ii) certains mycovirus réduisent l'agressivité de *P. viticola* de manière significative.
Un total de 27 souches monosporées de *P. viticola* ont été sélectionnées selon leur distribution le long d'un gradient d'agressivité, estimé par leur taux de sporulation (nombre de sporanges/mm2) sur feuilles de vigne en survie. Six jours après inoculation, les sporanges de ces souches ont été collectés pour réaliser une analyse du virome par RNAseq. Les résultats montrent un virome très riche, composé de 22 mycovirus au total avec des prévalences variables mais néanmoins élevées (30%-92% selon les virus). Cinq nouveaux virus ont été identifiés. Tous les isolats analysés ont été trouvés infectés par un minimum de deux virus, la moyenne étant de 11,6 +/- 3,9 virus/souche. On observe une grande variabilité dans la composition du virome des différentes souches, avec seulement deux souches sur 27 analysées qui présentent des viromes identiques. Aucun lien entre le nombre de mycovirus (richesse) et l'agressivité des souches n'a pu être observé. Par ailleurs, aucun mycovirus ne semble avoir d'effet fort sur le taux de sporulation des souches de mildiou de la vigne, même si la complexité des viromes conjuguée à un nombre limité de souches analysées complique l'interprétation des résultats.

Mots clés : Mycovirome - Hypovirulence - Mildiou - Vigne - Richesse.

First report of grapevine foveavirus A in the French vineyard

Isabelle Martin[1] (isabelle.martin@vignevin.com), Jean-Michel Hily[1], Wassim Rhalloussi[2]

[1] *LPA VitiViroBiome, Institut Français de la Vigne et Vin, Le Grau-du-Roi, France*
[2] *UMR A 1131 - SVQV, INRAE, Colmar, France*

More than 100 viruses have been identified in various *Vitis*worldwide. High throughput sequencing (HTS) largely contributed to this collection by allowing an unbiased identification of all sequences in a sample at a lower cost, becoming an inestimable tool for virus diagnostic in crops. The explosion of data revealed the complexity of grapevine virome, where mixed infection often rules. Although some species have been identified as important threat, like Nepoviruses or Grapevine red blotch virus an emerging virus; most of them have little to no known effect on grapevine.
Foveaviruses, belonging to the *Betaflexiviridae*family are flexuous shaped viruses with a ssRNA+ polyadenylated genome. The natural host range is restricted to a single or a few hosts, all woody crop plants like apple where infection can lead to detrimental symptoms. Among Foveaviruses, Grapevine rupestris stem pittingassociated virus, Grapevine Virus T, and the newly described Grapevine foveavirus A (GFVA) are capable of infecting grapevines. GFVA has only been described once in a survey of wild grapevine species in Switzerland (1). No symptom has been associated specifically to GFVA presence.
To assess the virome from unusual symptomatic plants observed in a vine cv. Pinot Gris in the Alsace region of France, we performed a ribodepletion-based HTS analysis that produced 61.1 million paired-end reads. In addition to the complex virome (Ampelovirus, Vitivirus, Trichovirus…), we reconstructed an 8.690 nts sequence sharing 91.75% identity with GFVA Genbank accession. Here, we present the first report of Grapevine foveavirus A in the French vineyard (2): diversity, pathogenicity and diagnostic technics will be discussed.
Thanks to HTS, virome description has never been more accurate compared to targeted diagnostic, but etiology of most emergent viral infection (like GFVA) remains unknown, efforts need to be placed in this direction.

Mots clés : Grapevine foveavirus A - Vitis vinifera - High throughput sequencing - Virome - Diagnostic.

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Investigations into the virome of grasses growing in the environment of sugarcane in Florida

Philippe Rott[1, 2] (philippe.rott@cirad.fr), Denis Filloux[1], Jean H. Daugrois[1], Emmanuel Fernandez[1], Charlotte Julian[1], Robert Beiriger[2], Calvin Odero[2], Sushma Sood[3], Philippe Roumagnac[1]

[1] *UMR PHIM, CIRAD, Montpellier, France*
[2] *Everglades Research and Education Center, University of Florida, Belle Glade, États-Unis*
[3] *Sugarcane Field Station, USDA-ARS, Canal Point, États-Unis*

Samples of 22 plant species and one aphid species (*Melanaphis sorghi*) were collected in the Everglades Agricultural Area (South Florida) from 2017-2019 to investigate the virome of grasses growing in the environment of sugarcane. Leaf samples were obtained from cultivated species such as *Saccharum* spp. (sugarcane), *Sorghum bicolor* (sorghum), *Zea mays* (maize), and wild species such as *Cynodon dactylon* (Bermuda grass), *Dactyloctenium aegyptum* (crowfoot grass), *Digitaria ciliaris* (Southern crabgrass), *Eleusine indica* (Goose grass), *Panicum dichotomiflorum* (Fall panicum), *Sorghum almum* (Columbus grass), *Stenotaphrum secundatum* (Saint Augustine grass), and *Urochloa platyphylla* (broadleaf signal grass). Detection and identification of the viruses present in 388 plant and 11 aphid samples was performed using the virion-associated nucleic acid (VANA)-metagenomics approach. Twenty-six viruses were identified, and 13 plant species and the aphid were infected by at least one virus. Fifteen of these 26 viruses were known viruses but 11 were putatively new virus species, including a marafivirus (*Tymoviridae*), a mastrevirus (*Geminiviridae*), a potyvirus (*Potyviridae*), and three sobemoviruses (*Solemoviridae*). The highest number of viruses was detected in Columbus grass and sorghum (9 and 13, respectively). *Potyvirus sacchari* (formerly *Sugarcane mosaic virus*, family *Potyviridae*) and *Waikavirus zeae* (formerly *Maize chlorotic dwarf virus*, family *Secoviridae*) were the most widespread virus species (9 and 7 plant species, respectively) and were also detected in the aphid. In a phylogenetic tree constructed with partial genome sequences (4000 nt) of *P. sacchari* obtained previously and herein, virus isolates from broadleaf signal gras, maize, sorghum, Southern crabgrass, sugarcane, and Saint Augustine grass were distributed in three different lineages, thus suggesting significant diversity of this virus in Florida. Only two *Saccharum*-infecting virus species, *P. sacchari* and *Polerovirus SCYLV* (*Sugarcane yellow leaf virus*), were found in sorghum and other grasses. These plants are, therefore, putative reservoirs for the causal agents of two sugarcane diseases in Florida.

Mots clés : Poaceae - Grasses - Sugarcane - VAVA sequencing - Virus diversity.

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Using TRV as a viral vector for genome editing in Prunus?

Amandine Bordat (amandinebordat@inrae.fr), Marielle Cochet, Sylvie German Retana, Véronique Decroocq

UMR BFP Equipe de Virologie, INRAE, Villenave-d'Ornon, France

In the frame of the TYPEX project, our objective is to optimize a protocol for efficient gene editing of the recalcitrant prunus species (stone fruit trees).
Tobacco rattle virus (TRV), a bipartite RNA virus, is a viral vector widely used in functional analysis of genes in different plant species. In particular, TRV was shown to provide an efficient tool to systemically deliver guide RNAs (gRNAs) for targeted genome modification in Cas9-expressing *Nicotiana benthamiana* (Ali *et al.,* 2015).
The genome of TRV-PEBV contains an additional subgenomic promoter sequence derived from pea early browning virus (PEBV), that allows the expression of the gRNA during the replication of the virus. Two others TRV-PEBV-derived vectors were recently developed: TRV_sgRNA_AtFT and TRV_sgRNA_AtLeu, where guide RNAs are fused in 3' to sequences that promote their mobility. Such modifications allow gRNAs to move to the shoot apical meristem and create more heritable gene editing in herbaceous plants (Ellison *et al.,* 2020).
Here, we aim at testing whether these vectors can be used to deliver gRNAs in *Prunus* species.
As a proof of concept, we first inoculated TRV_sgNbPDS3 and TRV_sgNbPDS_AtFT constructs targeting the phytoene desaturase gene *PDS* in *N. benthamiana*. Two to three weeks after agro-inoculation, patches of photobleaching appear in all the infected plants. After sowing the seeds collected from these plants, some of them were completely white indicating a heritable complete knockout of PDS.
The infectivity of those viral vectors in *Prunus persica* GF305 and *Prunus salicina* plums cv. Angeleno and Larry Ann was tested: TRV_sgRNA_AtLeu progeny was detected in GF305 and *P. salicina*cv. Angeleno plants after biolistic inoculation but not for TRV-PEBV. In order to improve the efficiency of the inoculation steps, we are currently optimizing agroinoculation methods of young seedlings and *in vitro* plants

Mots clés : VIGE - Prunus.

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Mise en place d'un MinION par un néophyte pour l'analyse de viromes de plantes

Patrick Cosson (patrick.cosson@inrae.fr), Chantal Faure, Bernard Bergey, Thierry Candresse, Armelle Marais

Equipe de virologie, UMR 1332 BFP, INRAE, Univ. Bordeaux, Villenave-d'Ornon, France

Les phytovirus menacent le rendement et la qualité des récoltes, situation aggravée par le changement climatique et la limitation des intrants en agriculture. Détecter ces virus est essentiel pour la gestion des maladies des cultures. L'utilisation des techniques de séquençage haut débit (HTS) permet d'explorer sans à priori le métagénome viral associé aux végétaux et d'étudier l'évolution de la diversité virale dans le temps et l'espace sous l'effet de différentes pressions de sélection.
Une étape clé est l'assemblage *de novo* des reads HTS en contig afin d'obtenir la séquence (quasi) complète de génomes viraux. La qualité de cet assemblage est impactée par la longueur des reads, le taux d'erreur et la profondeur du séquençage.
De nombreuses études ont été réalisées à partir de lectures courtes produites par séquençage Illumina™. La multiplication des études utilisant cette technologie via des plateformes de séquençage conduit à des délais significatifs dans l'acquisition des données.
Le développement des séquenceurs de troisième génération comme le MinION d'Oxford Nanopore Technologies permet de s'affranchir des plateformes. L'équipement est abordable, facile d'utilisation et génère des lectures longues. Il peut être déployé de façon flexible et au plus près du terrain.
Nous avons développé cette technologie au sein de notre laboratoire pour l'analyse de virome via le séquençage d'ARN bicaténaires purifiés, de l'acquisition des reads jusqu'à leurs traitements en utilisant des logiciels disponibles sur la plateforme d'analyse de données génomiques Galaxy ou le logiciel CLC Genomics Workbench (Qiagen). Nous avons comparé les virus détectés, la proportion de reads viraux, le nombre et la taille des contigs générés, la couverture des génomes avec les résultats obtenus par séquençage Illumina™. Nos résultats montrent des résultats similaires par les deux approches. Le MinION peut donc être utilisée par des néophytes pour avoir rapidement accès au virome d'un échantillon pour un coût raisonnable.

Mots clés : Métagénomique virale - Séquençage haut débit - MinION.

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Emergence du tomato fruit blotch virus (ToFBV) en Europe : premières descriptions dans le sud de la France

Eric Verdin (eric.verdin@inrae.fr), Jonathan Gaudin, Catherine Wipf-Scheibel

Unité de Pathologie végétale, INRAE, Avignon, France

Depuis son premier signalement en 2020, le tomato fruit blotch virus (ToFBV, *Blunervirus solani*) a été décrit sur plusieurs continents, en particulier dans des pays du sud de l'Europe. Les fruits de tomates infectées présentent des taches chlorotiques et un mûrissement irrégulier alors que les feuilles restent asymptomatiques. En raison de co-infections fréquentes avec d'autres virus de la tomate (ToBRFV, PepMV, TSWV notamment), des incertitudes demeurent sur le lien de causalité entre la présence exclusive du ToFBV et la symptomatologie qui lui est associée. De fortes suspicions désignent *Aculops lycopersici*, acarien Eriophyidae responsable de dégâts directs sur la tomate (acariose bronzée), comme le vecteur principal du ToFBV. La transmission du ToFBV par inoculation mécanique, par contact direct ou par la semence n'a pas été démontrée. L'analyse à posteriori d'échantillons anciens de tomate a démontré la présence du ToFBV, notamment en Italie dès 2012. L'émergence soudaine constatée dans les cultures de tomates pourrait être liée à la recrudescence récente de l'acariose bronzée suite à la suppression de méthodes de lutte chimique efficaces contre *A. lycopersici*.
En 2024, une enquête réalisée auprès des professionnels de la filière nous a permis de collecter des échantillons symptomatiques dans les régions du sud de la France afin de rechercher la présence du virus par analyses moléculaires et séquençage. Le ToFBV, jusque-là non décrit sur le territoire, a été détecté dans 6 départements des régions Provence-Alpes-Côte D'Azur, Occitanie et Nouvelle-Aquitaine (12 foyers au total). Sa présence a systématiquement été corrélée à de fortes pullulations d'*A. lycopersici*. Des études sont en cours afin d'évaluer la présence d'autres virus en co-infection, la diversité moléculaire des souches par séquençage de génomes complets, de préciser la gamme d'hôtes et les modes de transmission du virus, et de caractériser la sensibilité variétale chez la tomate.

Mots clés : ToFBV - Tomate - Acarien - Émergence.

Development of a sensitive Luminex xMAP-based microsphere immunoassay for specific detection of Rice Yellow Mottle Virus

Audrey Vernet[1] (audrey.vernet@ird.fr), Julie Bouillin[2], Nils Poulicard[1], Agnès Pinel-Galzi[1], Adeline Pinault[1], Guillaume Thaurignac[2], Martine Peeters[2], Ahidjo Ayouba[2], Séverine Lacombe[1]

[1] *PHIM, IRD, Montpellier, France*
[2] *TransVIH-MI, IRD, Montpellier, France*

Detected in more than 25 rice-growing countries, Rice yellow mottle virus (RYMV) is one of the most damaging viral pathogens devastating rice fields in Africa, causing up to 100% of yield loss. Very common approaches (DAS-ELISA, RT-PCR) for specific detection of RYMV from infected plants are available to date. However, a high-throughput screening method using a small amount of valuable samples was lacking.
Here we report the development of a high-sensitive Luminex xMAP-based microsphere immunoassay (MIA) for specific detection of RYMV. The working conditions of Luminex xMAP-based MIA was optimized by using appropriate antibody coupling, sample dilution, incubation time, concentration of biotin-labeled antibodies as well as the suitable ratio between the antibodies and the streptavidin-phycoerythrin (SAPE). Three monoclonal antibodies (MAbs) and one polyclonal antibody were used in this study. To set the cutoff for each antibody in our Luminex assay, we tested 47 RYMV-negative samples and 48-positive samples representing the full genetic diversity of RYMV in Africa (6 different strains from 8 African countries). A specificity of 97,87% was reached for the 4 antibodies. Sensibility varies from 54,17% to 97,92% with the highest level reached with the polyclonal antibody.
Under optimized conditions, the Luminex xMAP-based MIA was able to detect RYMV, no matter the antibody. Using polyclonal antibody, this technology allowed specific detection of RYMV with higher sensitivity (up to 500X) than conventional ELISA whereas samples infected by other plant virus species than RYMV (Maize streak virus or Rice stripe necrosis virus) were not detected.
This approach provides news perspectives in detecting RYMV in ancient or degrated samples, such as herbarium rice leaves from to the Department of «Muséum National d'Histoire Naturelle». This technology allowing multiplexing also extends its potential board usefulness in plant virus diagnosis for several rice viruses, with a true cost- and time-effective alternative to ELISA.

Mots clés : xMAP - MIA - Rice - RYMV - Diagnosis.

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