VIROID 2025

BOOK OF ABSTRACTS

Viroids and Viroid-like RNAs

Bari, 22-24 September 2025

INTERNATIONAL CONFERENCE VIROID 2025 – VIROIDS AND VIROID-LIKE RNAs

SEPTEMBER 22-24, 2025

"Aldo Moro" hall –
Law Department of the University of Bari Aldo Moro
Piazza Cesare Battisti, 1
BARI, ITALY

www.viroid2025.it
info@viroid2025

ORGANIZERS

BEATRIZ NAVARRO RAMIREZ
Institute for Sustainable Plant Protection (IPSP-CNR), Bari, Italy

KRITON KALANDITIS
Institute of Molecular Biology and Biotechnology, FORTH, University of Crete, Heraklion, Greece

SCIENTIFIC COMMITTEE

BEATRIZ NAVARRO RAMIREZ
IPSP-CNR, Bari, Italy

KRITON KALANDITIS
FORTH, University of Crete, Heraklion, Greece

MICHELA CHIUMENTI
IPSP-CNR, Bari, Italy

FRANCESCO DI SERIO
IPSP-CNR, Bari, Italy

LUISA RUBINO
IPSP-CNR, Bari, Italy

RITA MILVIA DE MICCOLIS ANGELINI
Di.S.S.P.A., Università degli Studi di Bari, Italy

ORGANIZING COMMITTEE

BEATRIZ NAVARRO RAMIREZ
IPSP-CNR, Bari, Italy

MICHELA CHIUMENTI
IPSP-CNR, Bari, Italy

MARIA ISABELLA PRICIGALLO
IPSP-CNR, Bari, Italy

NADIA SERALE
IPSP-CNR, Bari, Italy

OUR PARTNERS

PARTNERS

Conference secretariat:
Centro Italiano Congressi – CIC Sud (info@viroid2025), Piazza Cesare Battisti, 1 - 70122 Bari; phone: +39 080 5043737

SCIENTIFIC PROGRAM

DAY 1 MONDAY, September 22

8:30-9:30 Registration

9:30-9:40 Opening – Welcome to the event

9:40-10:20 OPENING LECTURE Viroids: The Archetype of subviral pathogens and RNA-regulators - *Detlev Riesner*

10:20-11:00 Coffee break

11:00-12:40 Session I Plant-viroid interaction and pathogenesis I (Chairs: Jernej Jakše – Purificación Lisón)

11:00-11:40 Keynote Lecture Understanding the interplay between PSTVd and plant defenses – *Kriton Kalantidis* (K1)

11:40-12:00 Hop latent viroid attenuates pathogenicity by suppressing RNA silencing machinery in cannabis and hop plants – *Charith Raj Adkar-Purushothama* (OC 1)

12:00-12:20 A master phosphoswitch relays plant immune signaling triggered by potato spindle tuber viroid – *Ying Wang* (OC2)

12:20-12:40 Apple scar skin viroid infection activates phloem-based defense response during infection – *Savita Chaudhary* (OC3)

12:40-15:00 LUNCH and poster session

15:00-17:10 Session II Diagnosis and epidemiology (Chairs: Dijana Škorić – Michael Hagemann)

15:00-15:20 Sequence variation of coconut cadang-cadang viroid in oil palm: insights from orange spotting symptom expression – *Nur Diyana Roslan* (OC4)

15:20-15:40 Towards field-ready detection of CBCVd in hop using CRISPR/Cas12a and RT-RPA– *Tanja Guček* (OC5)

15:40-16:00 Impact of brewing processes on the stability and infectivity of citrus bark cracking viroid in hops – *Sebastjan Radišek* (OC6)

16:00-16:30 Coffee break and poster session

16:30-16:50 Emerging viroid disease in 'Saiwaihong' apple in China and transcriptome analysis of dapple and scar apples – *Zhixiang Zhang* (OC7)

16:50-17:10 Citrus bark cracking viroid infecting pistachio (*Pistacia vera*) trees in Turkey – *Nihal Buzkan* (OC8)

17:30 Guided visit to the old city

DAY 2 TUESDAY, September 23

9:00-10:40 Session III Hepatitis delta like RNAs, ambiviruses and Obelisks (Chairs: Marcos de la Peña - Massimo Turina)

9:00-9:40 Keynote lecture Obelisks: Viroid-like colonists of human microbiomes – *Ivan. N. Zheludev* (K2)

9:40-10:00 Deltaviruses spread through a viral Trojan Horse – *Karim Majzoub* (OC9)

10:00-10:20 Fusarium graminearum ambivirus 1 requires ORF-B for replication and attenuates its fungal host pathogenicity – *Yi Guo* (OC10)

10:20-10:40 Woodchuck and deer Hepatitis Delta-like agents show distinct replication, innate immune activation and IFN-resistance compared to human Hepatitis D Virus - *Gnimah Eva Gnouamozi* (OC11)

10:40-11:20 Coffee break and poster session

11:20-13:00 Session IV Biodiversity of viroid-like RNAs: viroid-like RNAs emergence (Chairs: Shifang Li – Michela Chiumenti)

11:20-12:00 Keynote lecture: Discovery of novel plant viroids (*Pospiviroidae* family) and viroid-host associations through planetary data mining – *Marcos de la Peña* (K3)

12:00-12:20 Establishing a reverse genetic system for a viroid-like element from *Trichoderma spirale* – *Cristina Formiglia* (OC12)

12:20-12:40 Reservoirs of ancestral Deltaviruses found in water molds – *Massimo Turina* (OC13)

12:40-13:00 Identification and molecular characterization of viroid-like RNAs with coding capacity: zetaviruses and betaviruses – *Nadia Serale* (OC14)

13:00-14:00 LUNCH

14:00-14:40 Poster session (PhD students MSCA doctoral network project ViroiDoc)

14:40-17:50 Session V Plant viroid interaction and pathogenesis II (Chairs: *Charith Raj Adkar-Purushothama* – Ying Wang)

14:40-15:20 Keynote lecture Mechanisms of host adaptation mutations and characteristic symptoms in pospiviroids – *Teruo Sano* (K4)

15:20-15:40 Salicylic acid modulates volatile organic compound profiles during CEVd Infection in tomato plants – *Marc Balanzá* (OC15)

15:40-16:00 Antiviral immunity and suppression by RNA viruses – *Manfred Heinlein* (OC16)

16:00-16:20 Exploring extracellular vesicles (EVs) during viral and viroid infections – *Konstantina Katsarou* (OC17)

16:20-16:50 Coffee break and poster session

16:50-17:10 Small RNA-mediated host regulation in citrus bark cracking viroid-infected hop plants – *Michael Hagemann* (OC18)

17:10-17:30 Role of small RNAs in disease symptom recovery – *Laura Elvira González* (OC19)

17:30-17:50 Decoding hop–viroid interactions: a transcriptomic approach to CBCVd resistance – *Helena Volk* (OC20)

19:30 Social dinner at "*Corte di Torrelonga*" *(by bus)*

DAY 3 WEDNESDAY September 24

9:00-10:20 Session VI Replication and RNA structure (Chairs: Konstantina Katsarou – Beatriz Navarro)

9:00-9:40 Keynote lecture: Structural insights into the remodeled RNA polymerase II complex on RNA template – *Ying Wang (*K5)

9:40-10:00 Detection and characterization of viroid replication intermediates in hop using nanopore sequencing – *Jernej Jakše* (OC21)

10:00-10:20 Exploring RNA modifications in viroids: detection of N^6-methyladenosine (m6A) in citrus exocortis viroid – *Michela Chiumenti* (OC22)

10:20-11:00 Coffee break

11:00-12:00 Session VII Biotechnology (Chairs: José Antonio Daròs – Sebastjan Radišek)

11:00- 11:20 Harnessing citrus viroids as natural dwarfing agents for economic optimization in high-density citrus orchards – *Giorgios Vidalakis* (OC23)

11:20-11:40 Optimization of the viroid-double-self-splicing-intron system to produce recombinant RNA in *Escherichia coli* – *Diego Palpacelli* (OC24)

11:40-12:00 Synthetic circular RNAs and their potential use in plant protection – *Timo Schlemmer* (OC25)

12:00-12:30 Concluding remarks - *Francesco Di Serio*

LIST OF ORAL PRESENTATIONS

Opening Lecture

Viroids: The Archetype of subviral pathogens and RNA-regulators
D. Riesner, G. Steger

Keynote Lectures

Keynote Lecture 1 Understanding the interplay between PSTVd and plant defenses
K. Kalantidis

Keynote Lecture 2 Obelisks: Viroid-like colonists of human microbiomes
I. N. Zheludev, R. C. Edgar, M. J. Lopez-Galiano, M. de la Peña, A. Babaian, A. S. Bhatt, A. Z. Fire

Keynote Lecture 3 Discovery of novel plant viroids (*Pospiviroidae* family) and viroid- host associations through planetary data mining
O. Rueda, A. Cervera, A. Babaian, R. Chikhi, M. de la Peña

Keynote Lecture 4 Mechanisms of host adaptation mutations and characteristic symptoms in pospiviroids
T. Sano, Z. Zhang, S. Li

Keynote Lecture 5 Structural insights into the remodeled RNA polymerase II complex on RNA template
Y. Wang

Oral communications

OC1 Hop latent viroid attenuates pathogenicity by suppressing RNA silencing machinery in cannabis and hop plants
C. R. Adkar-Purushothama, K. Bouarab, J.-P. Perreault

OC2 A master phosphoswitch relays plant immune signaling triggered by potato spindle tuber viroid
Y. Wang

OC3 Apple scar skin viroid infection activates phloem-based defense response during infection
S. Chaudhary, P. Awasthi. Tamang, S. Kumar, S. Dhir, K. Sharma, V. Hallan

OC4 Sequence variation of coconut cadang-cadang viroid in oil palm: insights from orange spotting symptom expression
N. D. Roslan, S. Sundram, W. H. Lau, L. L. Kong, G, Vadamalai

OC5 Towards field-ready detection of CBCVd in hop using CRISPR/Cas12a and RT-RPA
T. Guček, S. Radišek

OC6 Impact of brewing processes on the stability and infectivity of citrus bark cracking viroid in hops
S. Radišek, M. Ocvirk, I.J. Košir, J. Jakše

OC7 Emerging viroid disease in 'Saiwaihong' apple in China and transcriptome analysis of dapple and scar apples
L. He, H. Xu, S. Li, Z., Zhang

OC8 Citrus bark cracking viroid infecting pistachio (*Pistacia vera*) trees in Turkey
N. Buzkan, S. C. Balsak, S. Karadağ, E. Türkmen, F. Aksu, A. Minafra

OC9 Deltaviruses spread through a viral Trojan Horse
J. McKellar, A. Fouillen, S. Lyonnais, F. Seigneuret, M-P. Blanchard, A. Trullo, L. Kumarasinghe, S. De Rossi, R. Sleiman, S. Desagher, R. Gaudin, H. de Rocquigny, S. Granier, J. Hepojoki, K. Majzoub

OC10 Fusarium graminearum ambivirus 1 requires ORF-B for replication and attenuates its fungal host pathogenicity
Y. Guo, M. Forgia, N. Serale, B. Navarro, V. Balmas, S. Oufensou, S. Daghino, A. Prodi, S. Schiwek, N. Miotti, M. Turina

OC11 Woodchuck and deer Hepatitis Delta-like agents show distinct replication, innate immune activation and IFN-resistance compared to human Hepatitis D Virus
G.E. Gnouamozi, Z. Zhang, s. Seitz, C . Lauber, S. Urban

OC12 Establishing a reverse genetic system for a viroid-like element from *Trichoderma spirale*
C. Formiglia, M. Forgia, B. Navarro, O. Rueda, F. Di Serio, N. Serale, S. Oufensou, V. Balmas, Q. Migheli, E. Gobbi, N. Miotti, F. Bono, M. de la Peña, M. Turina

OC13 Reservoirs of ancestral Deltaviruses found in water molds
M.J. López-Galiano, M. Forgia, O. Rueda, L. Botella, Z. Pasterny, A. Babaian, M. Turina, M. de la Peña

OC14 Identification and molecular characterization of viroid-like RNAs with coding capacity: zetaviruses and betaviruses
N. Serale, M. Chiumenti, P. Mussano, S. Rotunno, F. Venice, G. Della Rocca, M. Turina, F. Di Serio, A. Mello, L. Miozzi, B. Navarro

OC15 Salicylic acid modulates volatile organic compound profiles during CEVd Infection in tomato plants
M. Balanzá, F. Vázquez-Prol, I. Rodrigo, J.M. Bellés, F. Vera-Sirera, M.P. López-Gresa, P. Lisón

OC16 Antiviral immunity and suppression by RNA viruses
C. Huang, A. Sede, L. Elvira-Gonzalez, Y. Yan, M. Rodriguez, J. Mutterer, E. Boutant, L. Shan, M. Heinlein

OC17 Exploring extracellular vesicles (EVs) during viral and viroid infections
K. Katsarou, K. Kalantidis

OC18 Small RNA-mediated host regulation in citrus bark cracking viroid-infected hop plants
M. H. Hagemann, S. Jagani, U. Born, J. Jakše

OC19 Role of small RNAs in disease symptom recovery
L. Elvira-González, C. Matteoli, C. Himber, T. Blevins, V. Schurdi-Levraud, M. Heinlein

OC20 Decoding hop–viroid interactions: a transcriptomic approach to CBCVd resistance
H. Volk, S. Radišek, A. Čerenak, J. Jakše

OC21 Detection and characterization of viroid replication intermediates in hop using nanopore sequencing
A. Sečnik, H. Volk, N. Štajner, J. Jakše

OC22 Exploring RNA modifications in viroids: detection of N^6-methyladenosine (m6A) in citrus exocortis viroid
M. Chiumenti, P. Vopalensky, A. Škríba, L. Ďuričeková, A. Šimonová, O. Lukšan, F. Di Serio, B. Navarro, H. Cahova

OC23 Harnessing citrus viroids as natural dwarfing agents for economic optimization in high-density citrus orchards
I. Lavagi-Craddock, V. Lavagi, S. Bodaghi, G. Villalba-Salazar, S. Hajeri, J. Kaplan, M. Gómez, A. El-Kereamy, G. Vidalakis

OC24 Optimization of the viroid-double-self-splicing-intron system to produce recombinant RNA in *Escherichia coli*
D. Palpacelli, M. Spada, S. Pecchia, J.A. Daròs

OC25 Synthetic circular RNAs and their potential use in plant protection
M. Murr, A. Koch, T. Schlemmer

LIST OF POSTER PRESENTATIONS

P1 Symptom induction from non-infectious forms of citrus exocortis viroid in *Nicotiana benthamiana*
V. Aragonés, M. Eiras, J.A. Daròs

P2 Exploring the role of VIRP1, a bromodomain-containing protein crucial for pospiviroid infectivity
E. Bardani, K. Katsarou, C. Andronis, S. Ostendorp, J. Kehr, K. Kalantidis

P3 Double trouble: viral and viroid conspiracies in host cells
K. Katsarou, Z. Pentheroudaki, A. A. Nikoloudi, C. Spiridaki, N. Papadima-Karanikou, K. Kalantidis

P4 Molecular insights into viroid-induced defense mechanisms in grafted tomato plants
R. Marziale, M. Chiumenti, R. Spano, G. Bubici, A. Petrozza, F. Cellini, B. Navarro, F. Di Serio

P5 Effects of hop latent viroid infection on agronomic and biochemical traits of hops
T. G. Zambiassi, E.A.P. De Paula, C. Deschamps, R.R. Cipriano, K.S.S. Dias, A.F. Ramos, R.F. Calegario, M. Eiras

P6 Hop stunt viroid infecting fig (*Ficus carica* L.) trees in Türkiye
H. K. Ceren, N. Buzkan

P7 Characterization of citrus viroid VII population diversity across citrus hosts using amplicon sequencing
G.A. Chambers, A.D.W. Geering, D.R. Bogema, P. Holford, G. Vidalakis, N.J. Donovan

P8 First detection and molecular characterization of apple hammerhead viroid (AHVd) in Tunisian apple orchards
I. Hamdi, R. Soltani, A. Najar

P9 Unmasking the hidden virome of carob (*Ceratonia siliqua*)
A. Foteinou, A. C. Bibi, G. Papagiannakis, P. Topalis, D. Michelaki, P. Ioannidis, C. Bazakos, G. Emvalomatis, K. Kalantidis, K. Katsarou

P10 Varietal differences in symptom development of CBCVd infection in German hops
C. Krönauer, I. Raith, S. Euringer

P11 Uncovering viral and viroid diversity in crop–weed interactions in vegetables and citrus agroecosystems
N. Kryovrysanaki, G. Papadogiannakis, P. Topalis, K. Manousoudaki, K. Katsarou, C. Andronis, K. Kalantidis

P12 Investigating seed and pollen transmission of hop latent viroid in hops
M. Luigi, A. Taglienti, T. Ganino, M. Rodolfi, T. Lino, K. Carbone, F. Faggioli, L. Ferretti

P13 Rapid and field-deployable detection of viruses and viroids in tomato using LAMP directly from crude plant extracts
K. Manousoudaki, A. James, K. Katsarou, and K. Kalantidis, N. Kryovrysanaki

P14 Eradication of hop latent viroid (HLVd) in a medical cannabis seed bank using dot-blot hybridization and phytosanitary measures
P. Serra, G. Corrado, E. Llosa, J.A. Sanchez-Navarro, V. Pallás

P15 Detection of pospiviroids in solanaceous seed species collected from Africa
A. Skelton, A. Fowkes, L. Frew, J. Murphy, M.Mynett, O. Wagstaff, T.R. Pearce, C. Cockel, D.O. Nyamongo, J.M. Wasswa, A. Fox

P16 Chasing AHVd across Western Balkans – Croatian and Montenegrin examples
J. Zindović, V. Miljanić, N. Štajner, J. Jakše, J. Matijević, D. Škorić

P17 Virome analysis of Slovenian grapevines reveals high prevalence of GYSVd-1 and HSVd
V. Miljanić, J. Jakše, N. Štajner

P18 Armillaria ambiviruses: a potential tool for biocontrol of forest pathogens
B. Opelka, T. Tonka

P19 Ectopic expression and subcellular localization of the putative RNA-dependent RNA polymerase of Tulasnella ambivirus 4 in *Saccharomyces cerevisiae*
N. Serale, S. Daghino, M. Forgia, F. Di Serio, B. Navarro, L. Rubino

P20 New infectious agents in the Glomeromycotina: characterization of viroid-like elements in *Rhizophagus* and *Gigaspora* species
P. Mussano, M. Forgia, M. Chiumenti, S. Daghino, A. Crosino, B. Navarro, N. Serale, F. Di Serio, M. Turina, M. C. Garces-Ruiz, S. Declerck, L. Lanfranco

P21 Unveiling hidden ribozyme activities in fungal viroid-like RNAs
N. Serale, P. Mussano, M. Chiumenti, M. Forgia, S. Daghino, F. Di Serio, M. Turina, B. Navarro

P22 Fungal and plant RNA viruses found to exploit self-cleaving ribozymes for translation initiation
M.J. López-Galiano, O. Rueda, S. Chiba, M. Forgia, B. Navarro, A. Cervera, A. Babaian, F. Di Serio, M. Turina, M. de la Peña

P23 Designer circRNA$_{GFP}$ reduces GFP-abundance in Arabidopsis protoplasts in a sequence-specific manner, independent of RNAi pathways
M. Hossain, C. Pfafenrot, S.Nasfi, A. Sede, J. Imani, E. Šečić, M. Galli, P. Schäfer, A. Bindereif, M. Heinlein, M. Ladera-Carmona, K.H. Kogel

P24-DN1 Towards understanding viroid-host interactions through host gene disruption studies
J. Colaço, H. Volk, J-A. Daròs, N. Štajner, J. Jakše

P25-DN2 Climate change impact on viroid diseases. Application to Citrus bark cracking viroid (CBCVd)-infected hops
O. Lacroix, M. Mikulič Petkovšek, J. Jakše, S. Radišek

P26-DN3 Development of a disposable, selective, and sensitive electrochemical sensor for on-site detection of plant viroids
W.G. Liu, S. B. Hočevar, N. Tasić, S. Radišek, M. Fojta, A. Merkoçi

P27-DN4 *In vivo* imaging of viroid RNA and associated host factors
J. E. Lopez Ponce, L. Elvira-Gonzalez, T. Blevins, M. Heinlein

P28-DN5 CRISPR-based strategies for viroid detection: towards point-of-care diagnosis
Linh-Thi-Thuy Le, J.-A. Daròs

P29-DN6 Development of novel antiviroidal strategies: towards drug discovery
T. Stojkovska, F. Di Serio, K. Kalantidis, P. Lisón

P30-DN9 Viroid Affairs: Exploring the Hidden Network of RNA-Protein Interactions
V. Sánchez-Camargo, K. Katsarou, E. Bardani, L. Michailidou, S. Roussaki, H. van den Burg, K. Kalantidis

P31-DN10 RNA Sprays - Precision tools for the modulation of host genes to control viroid infections
L. Palatinus , A. Koch

P32 Immagina biotech
G. Marini

ABSTRACTS

Opening lecture

Viroids: The archetype of subviral pathogens and RNA-regulators

D. Riesner, G. Steger

Institute for Physical Biology, Heinrich-Heine-University, 20225 Düsseldorf, Germany.
E-mail: detlev.riesner@hhu.de

Ted Diener discovered in the early seventies that in some plants mid-size RNA without a capsid has the same biological phenomena like a complete, ten to hundred times larger virus. Not much later researchers in the US, Canada, Australia, and Germany detected those infectious RNAs in different plants, from ornamental plants to coconut palm trees. Heinz Sänger and colleagues in Germany described the viroid of the spindle tuber disease in potato as single-stranded, covalently closed circular RNA molecules existing as highly base-paired rod-like structures. Not only a new type of pathogen but also a new type of RNA, i.e. the circular was found in Nature. Many years later circular RNA was detected also as regulators in normal cells. Since viroids do not code for any protein, the replication is carried out completely by cellular factors either in the nucleus or in the chloroplast depending on the viroid species. The trick, that one and the same viroid molecule can assume different metastable structures for different functions, was later found in many RNAs. Work on viroids initiated development of new methods, gel electrophoretic analytics and chromatographic purification. Chromatography in form of easily handable kits is used today worldwide for nucleic acid purification in molecular biology. Even the covalent nature of hydrogen bonds was proven in viroids. When viroids were also discussed as subviral agents of neurodegenerative diseases like scrapie in sheep and CJD in humans, the systematic comparison of viroid features with those of the agents of neurodegeneration showed - although disbeleaved for many years – that nature has evolved also replicating proteins, the Prions. What started with viroids in plants, developed today as the prion concept to understand Alzheimer´s and other neurodegenerative diseases.

Keynote lecture 1

Understanding the interplay between PSTVd and plant defenses

K. Kalanditis

Institute of Molecular Biology and Biotechnology, FORTH, Heraklion, Greece 2. Dept. of Biology, University of Crete, Heraklion, Greece.
E-mail: kriton@imbb.forth.gr

Viroid replication and movement depend on a complex interplay of host factors and defense pathways. Previously, we demonstrated the interaction of viroid biology with silencing pathways. While DCL4 predominantly processes *Pospiviroidae* RNAs, alternative pathways contribute significantly to antiviral defense. Using CRISPR/Cas9-generated DCL knockouts and plants impaired in microRNA biogenesis, we show that multiple silencing routes act in parallel. According to current models, an RNase III endonuclease is required to cleave the double-stranded RNA concatemer intermediates produced during replication. Plants possess two types of RNase III endonucleases: DCLs (Dicer-Like) and RTLs (RNase Three-Like), with RTLs being the least studied. To investigate RTL function in the PSTVd biological cycle, we identified all putative RTLs in the *Nicotiana benthamiana* transcriptome and suppressed their expression. RTL levels were first transiently reduced through Virus-Induced Gene Silencing (VIGS), followed by the generation of stable knockdown plants expressing a hairpin RNA (hpRNA) targeting RTL sequences. In parallel, CRISPR/Cas9 technology was used to create RTL knockout plants. Infectivity studies in these lines will clarify the role of host RTLs in viroid biogenesis.
In addition, we elaborate on the role of VIRP1, a bromodomain protein previously shown to be essential for viroid infectivity. We found that VIRP1 promotes liquid–liquid phase separation, and that mutations in its bromodomain disrupt condensate formation and reduce PSTVd accumulation. These findings suggest that VIRP1 contributes to the subnuclear localization and trafficking of viroid RNA.
Together, our findings establish complementary genetic and molecular tools and point to an integrated model in which RNA silencing, nucleic acid processing enzymes, and RNA-binding proteins collectively determine the outcome of viroid infection.

Keynote lecture 2

Obelisks: Viroid-like colonists of human microbiomes

I. N. Zheludev[1], R. C. Edgar[2], M. J. Lopez-Galiano[3], M. de la Peña[3], A. Babaian[4,5], A. S. Bhatt[6,7], A. Z. Fire[6,8]

[1]Stanford University, Department of Biochemistry, Stanford, CA, USA; [2]Independent researcher, Corte Madera, CA, USA; [3]Instituto de Biología Molecular y Celular de Plantas, Universidad Politécnica de Valencia–CSIC, Valencia, Spain; [4]University of Toronto, Department of Molecular Genetics, Ontario, Canada; [5]University of Toronto, Donnelly Centre for Cellular and Biomolecular Research, Ontario, Canada; [6] Stanford University, Department of Genetics, Stanford, CA, USA; [7]Stanford University, Department of Medicine, Division of Hematology, Stanford, CA, USA; [8]Stanford University, Department of Pathology, Stanford, CA, USA.
E-mail: Ivan.zheludev@gladstone.ucsf.edu

Previously, we described "Obelisks," a class of heritable RNA elements sharing several properties: (1) apparently circular RNA ∼1 kb genome assemblies, (2) predicted rod-like genome-wide secondary structures, and (3) open reading frames encoding a novel "Oblin" protein superfamily. A subset of Obelisks includes a variant hammerhead self-cleaving ribozyme. Obelisks form their own phylogenetic group without detectable similarity to known biological agents. Surveying globally, we identified 29,959 distinct Obelisks (clustered at 90% sequence identity) from diverse ecological niches. Obelisks are prevalent in human microbiomes, with detection in ∼7% (29/440) and ∼50% (17/32) of queried stool and oral metatranscriptomes, respectively. We established *Streptococcus sanguinis* as a cellular host of a specific Obelisk and found that this Obelisk's maintenance is not essential for bacterial growth. Our observations identify Obelisks as a class of diverse RNAs of yet-to-be-determined impact that have colonized and gone unnoticed in human and global microbiomes. We are now beginning to study Obelisks in the context of their cellular hosts, their larger ecological roles, and their potential impacts on human and environmental health. This *research-in-progress* presentation will cover *in vitro* biochemical characterization, microbial phenotyping, and bacterial single-cell RNA sequencing of the model *S. sanguinis*- Obelisk system - and how these new findings impact our nascent understanding of Obelisk molecular biology.

Keynote lecture 3

Discovery of novel plant viroids (*Pospiviroidae* family) and viroid-host associations through planetary data mining

O. Rueda[1], A. Cervera[1], A. Babaian[2], R. Chikhi[3], M. de la Peña[1]

[1]Instituto de Biología Molecular y Celular de Plantas, UPV-CSIC, Valencia, Spain; [2]Department of Molecular Genetics, University of Toronto, Toronto, Canada; [3]Institut Pasteur, Paris, France.
E-mail: rivero@ibmcp.upv.es

Viroids are the tiniest infectious genomes ever described, consisting of just 246-401 nt of non-coding circular RNA (circRNA) with uncertain evolutionary origins. Since their discovery in potato plants in 1971, viroids have been considered rare biological entities, with only 45 species reported in flowering plants over the past half-century. Recent studies, however, have challenged this view by revealing thousands of novel viroid-like entities distributed across a wide range of ecological niches. Intriguingly, most of these newly discovered circRNAs appear to replicate in much simpler organisms, including both eukaryotes and prokaryotes, rather than plants. In this study, we conducted a comprehensive bioinformatic search for classical plant viroids (family *Pospiviroidae*, 40 known species) using Logan, a planetary-scale genetic database. Through structure-based analysis, we identified 13,000 genomes fulfilling the features of this viroid family: a small circRNA sequence, rod-like secondary structure, and the presence of the central and terminal domains conserved in all members. Among these, we identified up to 20 divergent genomes that tentatively represent novel plant viroid species. These findings represent a ~50% increase in the known diversity of the family *Pospiviroidae* and suggest a broader plant host range than previously known. Our results expose that plant viroids can be detected in previously unreported species of angiosperms, spanning from herbaceous, like black pepper or Chinese cucumber, to woody plants, including bamboo, poplars or magnolias. This expansion will help us better understand the biology of these minimal agents, but it also underscores the more limited genetic diversity and abundance of typical plant viroids compared to other minimal circRNA entities, such as obelisks and viroid-like RNAs with ribozymes.

Keynote lecture 4

Mechanisms of host adaptation mutations and characteristic symptoms in pospiviroids

T. Sano[1], Z. Zhang[2], S. Li[2]

[1]Plant Pathology Laboratory, Faculty of Agriculture and Life Science, Hirosaki University, Bunkyo-cho 3, Hirosaki 036-8561, Japan; [2]State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection (IPP), Chinese Academy of Agricultural Sciences (CAAS), Beijing, China.
E-mail: sano@hirosaki-u.ac.jp

Host adaptive mutations and RNA silencing in hop stunt viroid

Hop stunt disease first appeared in Japan in the 1940s and became epidemic in domestic hop growing regions by the late 1980s. The causal agent is hop stunt viroid (HSVd), and various variants have since been found in fruit trees such as grapevine, citrus, plum, peach and others around the world. When these HSVd variants were infected to hop, they each developed mild to severe hop stunt symptoms and generated host adaptation mutations specific to each variant; particularly the grapevine variant underwent sequential mutations at five positions (25, 26, 54, 193, and 281) in the 297 nucleotides genome over a 15-year persistent infection period, and eventually converging to a dominant variant in which all five nucleotides were mutated. This mutation perfectly matched the dominant form in hop stunt disease-endemic regions, demonstrating that the cultivated grapevine variant was the origin of hop stunt disease in Japan. Competitive infection assays revealed that hop-adapted variants did not have significant advantages in infectivity or propagation in hops, but showed the advantage of being genetically more stable than the grapevine variant. Analysis of viroid-derived small RNA (HSVd-sRNA) revealed that the number of reads of HSVd-sRNA derived from the regions containing the five mutations were reduced, suggesting that the hop-adapted mutations reduce the selective pressure for RNA silencing. The order of successive mutations was first 54, then 281, 25, 26, and finally 193. That is, it was hypothesized that the initial hop adaptive mutation occurred at position 54 to escape from the attack of RNA silencing, which subsequently induce further secondary adaptive mutations to overcome the newly emerged structural constraints or attacks of RNA silencing, ultimately resulting in all five positions being altered and falling into a metastable state.

A possible mechanism of the development of severe necrosis incited by potato spindle tuber viroid infection.

'Moneymaker' tomato is susceptible to potato spindle tuber viroid (PSTVd) infection, and only develops mild leaf curling. However, knockdown of DCL2 and DCL4, key factors in RNA silencing, rendered the plant highly sensitive, resulting in a more than two-fold increase in PSTVd accumulation, severe dwarfing, and severe yellowing and necrosis of the leaves. Small RNA analysis of necrotic stems and leaves revealed that in the DCL2&4 knockdown lines, expression levels of the stress-responsive miR398 and miR398a-3p were approximately 8–9-fold higher than in the wild type. Expression of copper/zinc (Cu/Zn) superoxide dismutase (SOD), which scavenges reactive oxygen species (ROS), was significantly reduced, and ROS production and ROS scavenging were approximately 3-fold and 1.5-fold higher, respectively. Namely, in the DCL2&4-knockdown 'Moneymaker', the rapid increase of PSTVd titer could not be suppressed due to a reduced defense ability of DCL2 and DCL4, and leading to abnormally high expression of stress-responsive miR398 and miR398a-3p. As a result, excessive ROS production due to PSTVd infection cannot be controlled, resulting in the onset of severe necrosis, a typical symptom appeared on tomato plants infected with virulent strains of PSTVd.

Keynote lecture 5

Structural insights into the remodeled RNA polymerase II complex on RNA template

Y. Wang

Department of Plant Pathology, University of Florida, USA
E-mail: ying.wang1@ufl.edu

DNA-dependent RNA polymerases recognize not only DNA templates but also RNA templates. This RNA-dependent RNA polymerase activity was exploited by human Hepatitis delta virus in mammalian cells and viroids in plants. Accumulating evidence suggested that distinct transcription complexes function on DNA templates and potato spindle tuber viroid (PSTVd) RNA template, an understudied layer of transcriptional regulation. Here, we provided evidence demonstrating the reorganization of 12-subunit Pol II to 7-subunit for PSTVd transcription in vivo. Rpb4, Rpb5, Rpb6, Rpb7, and Rpb9 are not involved in PSTVd transcription. A splicing variant of the transcription factor IIIA with seven zinc finger domains (TFIIIA-7ZF) aids the remodeled Pol II for transcribing PSTVd. Based on AlphaFold3 prediction, we obtained the structure of the remodeled Pol II with PSTVd RNA and TFIIIA-7ZF. The predicted structure and experimental data all showed that TFIIIA-7ZF binds to the left terminal domain of PSTVd. The C-terminus of TFIIIA-7ZF is predicted to interact with Rpb2, which is confirmed by the observation that C-terminus-deleted TFIIIA-7ZF lost Pol II binding ability. Altogether, the data provide the structure insights into the remodeled Pol II and TFIIIA-7ZF transcription complex on RNA template.

Oral communication 1

Hop latent viroid attenuates pathogenicity by suppressing RNA silencing machinery in cannabis and hop plants

C. R. Adkar-Purushothama[1], K. Bouarab[2], J.-P. Perreault[1]

[1]RNA Group/Groupe ARN, Département de Biochimie et de génomique fonctionnelle, Faculté de Médecine et des Sciences de la Santé, Pavillon de Recherche Appliquée au Cancer, Université de Sherbrooke, Sherbrooke, Québec, J1E 4K8, Canada; [2]Centre SEVE, Département de Biologie, Faculté des Sciences, Université de Sherbrooke, 2500 Boulevard de l'Université, Sherbrooke, Québec, J1K2R1, Canada.
E-mail: charith.adkar@usherbrooke.ca

Recently, hop latent viroid (HLVd) has attracted significantly more attention from both academic and industrial researchers than before due to its impact on cannabis cultivation in North America. Although most HLVd-infected plants remain asymptomatic, HLVd infection has resulted in both the yield and a loss of quality. By performing genome-wide analysis of HLVd against hop and cannabis genomes, we have identified small RNAs derived from HLVd that potentially target the host RNAs that are known to be involved in the plant's RNA silencing machinery, such as double-stranded RNA-binding protein 3 (DRB3), and putative nuclear RNA export factor SDE5. Of these targets, the former is known to initiate primary RNA silencing, whereas the latter is known to be involved in the *trans*-acting small-interference RNA (tasiRNA) pathway. *In silico* analysis was validated by artificial microRNA experiment and single and double mutagenic studies. This study deepens our understanding of long-standing questions such as (i) how HLVd is asymptomatic in the majority of infected plants, and (ii) how double infection of HLVd with Citrus bark cracking viroid (CBCVd) results in severe disease symptoms in hops, and, more importantly (iii) how viroids escape the RNA silencing mechanism.

Oral communication 2

A master phosphoswitch relays plant immune signaling triggered by potato spindle tuber viroid

Y. Wang

Department of Plant Pathology, University of Florida, USA
E-mail: ying.wang1@ufl.edu

Host immunity, which is essential for survival, distinguishes self and nonself molecules in order to detect invading pathogens and subsequently activates defense. The mechanisms for host immune machinery to sense major types of pathogens are more or less understood, except for viroids. In plants, the coordinated PTI (pathogen-triggered immunity) and ETI (effector-triggered immunity) modulate the detection of bacteria, fungi, and viral pathogens and the activation of defense-related genes. A series of phosphorylation cascades are often found underlying the immune signaling. For viroids, which are noncoding RNA-based plant pathogens, it is clear that their infection can trigger the activation of immunity, but it remains unclear how the immunity signaling is regulated. Based on our recent proteomic analysis, we identified a key phosphorylation in a conserved site within RPM1-interacting protein 4 (RIN4). This site is highly phosphorylated upon the infection of potato spindle tuber viroid (PSTVd), which consequently activates the downstream marker genes. The significance of this funding will be further discussed in the context of plant-viroid interactions.

Oral communication 3

Apple scar skin viroid infection activates phloem-based defense response during infection

S. Chaudhary[1,2], P. Awasthi[1], A. Tamang[1,2], S. Kumar[1], S. Dhir[3], K. Sharma[4], V. Hallan[1,2]

[1]Plant Virology Laboratory, Division of Biotechnology, CSIR—Institute of Himalayan Bioresource Technology, Palampur 176061, Himachal Pradesh, India; [2]Academy of Scientific and Innovative Research (AcSIR), Ghaziabad 201002, India; [3]Molecular Plant Virology Group, R&D Lab, Department of Bio-Sciences & Technology, Maharishi 5 Markandeshwar (Deemed to be University), Mullana, Ambala-133207 HR India; [4]Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyala, HPAU, Palampur, Himachal Pradesh, India.
E-mail: savita.ihbt20a@acsir.res.in; hallan@ihbt.res.in

Viroids are the smallest known infectious, non-coding, circular single-stranded RNA molecules (234–401 nt) that can infect various plant hosts, replicate independently, and cause significant damage to economically important crops worldwide. The apple scar skin viroid (ASSVd) is responsible for causing apple scar skin and dappling disease in apples, as well as pear rusty skin, pear fruit crinkle, and pear fruit dimple diseases. ASSVd is a circular RNA molecule consisting of about 330 nucleotides of single-stranded RNA that can replicate autonomously within the nucleus of host cells. A recent survey of ASSVd from Himachal Pradesh showed a notable incidence of 72.9%, which is much higher than previous reports in Indian orchards (27.6%). The rising prevalence of these pathogens, especially viroids, is a major concern.

Viroids rely entirely on host factors for their pathogenicity, making it essential to study the host proteins that interact with ASSVd RNA for effective control strategies. Phloem protein 2 (PP2) from host sap (cucumber) was found to interact with ASSVd RNA. This interaction was confirmed through electrophoretic mobility shift assays and north-western assays. When PP2 from plants was artificially added to whiteflies' diet with viroid, it was found to increase ASSVd transmission. Interestingly, mutagenesis studies on AtPP2-A1 from *Arabidopsis thaliana* indicated that mutations in AtPP2-A1 enhanced the systemic transport of ASSVd. The mutant lines of AtPP2-A1 showed greater susceptibility to ASSVd and cucumber mosaic virus infection, suggesting a role in defense mechanisms. These findings strongly suggest that AtPP2-A1 is involved in phloem-based defense against viroid infection.

Oral communication 4

Sequence variation of coconut cadang-cadang viroid in oil palm: insights from orange spotting symptom expression

N. D. Roslan[1], S. Sundram[1], W.H. Lau[2], L.L. Kong[3], G. Vadamalai[2,3]

[1]Malaysian Palm Oil Board, 6, Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia; [2]Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia; [3]Institute of Plantation Studies, Universiti Putra Malaysia, Malaysia.
E-mail: diyana@mpob.gov.my

Coconut cadang-cadang viroid (CCCVd) is associated with orange spotting (OS) disorder in oil palm (*Elaeis guineensis*) in Malaysia, yet the relationship between CCCVd sequence variation and OS symptomatology remains unclear. In this study, CCCVd variants from symptomatic field palms ($CCCVd_{OP}$) were characterized using RT-PCR, cloning, sequencing, and qPCR quantification. Three distinct variants of various nucleotide length ($CCCVd_{OP246}$, $CCCVd_{OP251}$, and $CCCVd_{OP279}$) were identified, with $CCCVd_{OP246}$ the most prevalent. Despite high sequence similarity (>93%), the variants displayed nucleotide differences affecting multiple viroid domains. Notably, OS symptom severity did not correlate with viroid titer or palm age. The coexistence of multiple variants in single palms supports a quasi-species model and may contribute to symptom heterogeneity. This is the first report linking sequence diversity of CCCVd to symptom variation in field-infected oil palms, offering foundational insight into viroid-host interactions in perennial crops.

Oral communication 5

Towards Field-Ready Detection of CBCVd in Hop Using CRISPR/Cas12a and RT-RPA

T. Gučeк[1] and S. Radišek[2]

[1]Slovenian Institute for Hop Research and Brewing, Cesta Žalskega tabora 2, 3310 Žalec, Slovenia;
[2]Slovenian Institute for Hop Research and Brewing, Cesta Žalskega tabora 2, 3310 Žalec, Slovenia.
E-mail: tanja.gucek@ihps.si; sebastjan.radisek@ihps.si

Cocadviroid rimocitri (citrus bark cracking viroid, CBCVd) causes an incurable severe hop stunt disease, which affected nearly 500 ha (out of 1500 ha) of hop fields in Slovenia between 2007 and 2022. In previous research, we developed sensitive molecular methods that allow multiplex detection of several viroids in hops. The development of new methods is a necessary step towards detection of new outbreaks and limitation of further spread. A promising approach in plant diagnostics is the use of the CRISPR/Cas system, which has been developed to identify a number of pathogens due to its high sensitivity, speed and ease of use. The CRISPR/Cas system, when combined with pre-amplification of the target using RPA (recombinase polymerase amplification) and lateral flow assays, enables the detection of viroids in the field in less than one hour. With the aim of developing a CRISPR/Cas12a-RT-RPA diagnostic method for the field detection of CBCVd, we designed several different crRNAs and RT-RPA primers. Our primary goal is to detect CBCVd without the need for RNA isolation, therefore we tested various methods for homogenization of plant tissue using different buffers and homogenization tools. We successfully confirmed the CBCVd in the crude extract using RT-PCR and RT-qPCR. We then optimized the CRISPR/Cas12a-RT-RPA reaction with fluorescently labelled ssDNA probes in a qPCR instrument. We plan to test lateral flow assays in combination with probes ladled with biotin to enable field use. The development of a method that allows sensitive and fast detection, without prior RNA isolation, while being suitable for field use, will represent a major advancement in hop research as well as plant diagnostics in general.

Oral communication 6

Impact of brewing processes on the stability and infectivity of citrus bark cracking viroid in hops

S. Radišek[1], M. Ocvirk[1], I. J. Košir[1], J. Jakše[2]

[1]Slovenian Institute for Hop Research and Brewing, Cesta Žalskega tabora 2, 3310 Žalec, Slovenia; [2]University of Ljubljana, Biotechnical Faculty, Department of Agronomy, Jamnikarjeva 101, SI-1000 Ljubljana.
E-mail: sebastjan.radisek@ihps.si

Citrus bark cracking viroid (CBCVd), recently reclassified taxonomically as *Cocadviroid rimocitri*, represents a major threat to *Humulus lupulus* (hop) cultivation, particularly in regions such as Slovenia and Germany where extensive outbreaks have been reported. Management primarily relies on the removal and destruction of infected plants; however, the long incubation period can result in asymptomatic infected plants being harvested. As hop is a key ingredient in the brewing industry, concerns have arisen regarding the stability of CBCVd in processed hop material and the potential for viroid transmission through brewing residues. This study investigated the survival of CBCVd in infected hop cones subjected to various brewing stages, including kettle hopping and dry hopping during fermentation and maturation. Using RT-PCR, we confirmed the presence of CBCVd in fresh hop cones even after boiling, as well as in dry-hopping treatments—particularly in lower-extract worts and under cold maturation conditions. However, infectivity assays revealed that, despite detectable levels of CBCVd, spent hops failed to transmit the viroid to healthy plants, indicating a low risk of spread through brewing waste. These findings underscore the importance of proper hop waste disposal, while also providing reassurance to both the agricultural and brewing sectors regarding the limited infectivity of CBCVd in hop cones following brewing processes.

Oral communication 7

Emerging viroid disease in 'Saiwaihong' apple in China and transcriptome analysis of dapple and scar apples

L. He[1], H. Xu[1], S. Li[1,2], Z. Zhang[1]

[1]Institute of Plant Protection, Chinese Academy of Agricultural Sciences (CAAS), Beijing, China; [2]Center for Biosafety, Chinese Academy of Inspection and Quarantine, Sanya, Hainan Province, China.
E-mail: zhangzhixiang02@caas.cn

Apple scar skin viroid (ASSVd) infection induces apple scar skin disease that is a major threat to apple production in China due to the reduced yield, poor fruit quality, and, in severe cases, complete loss of marketable value. Despite being discovered nearly a century ago, the disease remains a persistent challenge with incidence of over 60% in certain orchards. Recently, a novel disease affecting small immature fruits has surfaced in 'Saiwaihong' apples (*Malus pumila*), a new variety widely cultivated across over 20,000 hectares. The detection of 152 symptomatic and 122 asymptomatic fruit samples using RT-PCR and dot-blotting hybridization found a close association between the disease and the presence of ASSVd. These observations emphasize the significance of ongoing monitoring for ASSVd, both in its familiar forms and potential new variants.
In addition, to explore the molecular basis of the ASSVd-induced diseases, transcriptome analysis was performed for 'Fuji' apples showing dapple symptoms collected at an early stage of fruit coloring (in early September) and 'Venus Golden' apples showing scar skin symptoms collected at the early stage of fruit development (in June). The results showed that anthocyanin biosynthesis pathway was down-regulated in dapple apples and lignin biosynthesis pathway was up-regulated in scar apple. Both pathways belong to the phenylpropanoid metabolite pathway. Thus, the diseases should be associated with the regulation of phenylpropanoid metabolite pathway by ASSVd infection.

Oral communication 8

Citrus bark cracking viroid infecting pistachio (*Pistacia vera*) trees in Turkey

N. Buzkan[1], S. C. Balsak[1], S. Karadağ[2], E. Türkmen[1], F. Aksu[3], A. Minafra[4]

[1]Plant Protection Department, Agriculture Faculty, Kahramanmaraş Sütçü Imam University, Kahramanmaraş, Türkiye; [2]ARIPEN Ltd., Birecik, Şanlıurfa, Türkiye; [3]Biology Department, Science Faculty, Kahramanmaraş Sütçü Imam University, Kahramanmaraş, Türkiye; [4]Institute for Sustainable Plant Protection - National Research Council of Italy (IPSP-CNR).
E-mail: nbuzkan@ksu.edu.tr; nbuzkan@gmail.com

Pistachio is an important crop worldwide as well as in Turkey. Citrus bark cracking viroid (CBCVd) (family *Pospiviroidae*, genus *Cocadviroid*), formerly known as Citrus viroid IV, has been known as a causative disease agent in citrus and hops. After discovery of CBCVd in pistachio trees by high throughput sequence in Northern California in 2018, this study investigated the presence of the viroid in pistachio plantations in Southeastern Anatolia, which is a major pistachio growing area. Approx. 19% of 218 tested pistachio trees (*Pistacia vera*) bearing symptoms which have not been yet associated with any known pathogen, was found to be infected with CBCVd. Full length genome sequences of different variants ranging from 282 to 286 nucleotides had considerable sequence variation with 14 distinct haplotypes, resulting in a haplotype diversity of 0.925. Overall, the estimated average number of nucleotide differences (k) and nucleotide diversity (π) were 5.16 and 0.019, respectively. These findings indicate that the CBCVd in tested pistachio trees contained a heterogeneous population. The nucleotide homology among the Turkish CBCVd-pistachio isolates was between 96-100%. The sequences were most closely related the isolate W11 from pistachio (MF198463.1). CBCVd-pistachio isolates were both mechanically transmitted onto *Cucumis sativus* and grafted onto widely used rootstocks. Symptom expression was recorded on herbaceous indicators after 20 days of post-inoculation. Graft transmissibility of CBCVd was checked with RT-PCR.

This research was granted by TUBITAK (#221O411).

Oral communication 9

Deltaviruses spread through a viral Trojan Horse

J. McKellar[1], A. Fouillen[2], S. Lyonnais[3], F. Seigneuret[4], M-P. Blanchard[5], A. Trullo[1], L. Kumarasinghe[6], S. De Rossi[5], R. Sleiman[1], S. Desagher[6], R. Gaudin[6], H. de Rocquigny[4], S. Granier[2], J. Hepojoki[7], K. Majzoub[1]

[1]Institut de Génétique Moléculaire de Montpellier (IGMM), Université de Montpellier (UM), Montpellier and CNRS, France; [2]Institut de Génomique Fonctionnelle (IGF), CNRS, Montpellier, France; [3]Centre d'Études des Maladies Infectieuses et Pharmacologie Anti-Infectieuse (CEMIPAI), CNRS, Montpellier, France; [4]Morphogenèse et Antigénicité du VIH et des Virus des Hépatites (MAVIVH), Inserm, Tours, France; [5]Montpellier Ressources Imagerie (MRI), CNRS, Montpellier, France; [6]Institut de Recherche en Infectiologie de Montpellier (IRIM), CNRS UMR9004, Université de Montpellier, Montpellier, France; [7]University of Helsinki, Medicum, Department of Virology, Helsinki, Finland.
E-mail: karim.majzoub@igmm.cnrs.fr

Hepatitis D-like viral satellites, known as deltaviruses, have been recently discovered in a wide range of animals. These viruses are thought to expropriate glycoproteins from helper viruses to form infectious particles. Here, we challenge this paradigm and demonstrate that deltaviruses are packaged within helper virus particles, using them as viral Trojan Horses for cell entry. By leveraging orthogonal electronic and photonic super-resolution microscopy, we visualize deltaviruses enclosed within virions from rhabdo-, herpes-, and arenavirus families. We show that this conserved hitchhiking mechanism ensures concomitant deltavirus-helper virus spread, which advantages the dissemination of deltaviruses, broadens their host range and expands their tropism. Our findings reveal a previously unrecognized mode of viral transmission, providing a framework to investigate overlooked deltavirus infections outside of the human liver.

Oral communication 10

Fusarium graminearum ambivirus 1 requires ORF-B for replication and attenuates its fungal host pathogenicity

Y. Guo[1], M. Forgia[1], N. Serale[2], B. Navarro[2], V. Balmas[3], S. Oufensou[3], S. Daghino[1], A. Prodi[4], S. Schiwek[5], N. Miotti[1], M. Turina[1]

[1]Institute for Sustainable Plant Protection, National Research Council of Italy, Strada delle Cacce 73, 10135, Torino, Italy; [2]Institute for Sustainable Plant Protection, National Research Council of Italy, Via Amendola 165/A, 70126, Bari, Italy; [3]Department of Agricultural Sciences and NRD—Desertification Research Center, University of Sassari, Viale Italia 39a, 07100 Sassari, Italy; [4]Department of Agricultural and Food Sciences, University of Bologna, Viale Fanin 40, 40127, Bologna, Italy; [5]Julius Kühn-Institute, Institute for Plant Protection in Field Crops and Grassland, Messeweg 11/12, 38104 Braunschweig, Germany.
E-mail: yiguo@cnr.it

Ambiviruses are a recently defined class of mycoviruses with circular single-stranded RNA genomes, exhibiting both viroid-like and viral features. Their genomes encode an RNA-dependent RNA polymerase (RdRp) in one polarity orientation and possess paired self-cleaving ribozymes in both sense and antisense orientations. They typically encode two conserved open reading frames (ORFs) in ambisense orientation: ORF-A encodes an RdRp, while the function of ORF-B remains unknown. Here, we report the construction of the first infectious cDNA clone of an ambivirus, Fusarium graminearum ambivirus 1 (FgAV1), using a head-to-tail dimer placed downstream of a fungal promoter. A monomeric cDNA clone was used as a negative control. The dimer construct successfully initiated replication in virus-free *F. graminearum*, as evidenced by the accumulation of negative-sense circular RNA, while the monomer did not. FgAV1 was horizontally transmitted via hyphal anastomosis to virus-free *F. graminearum* recipient strains. Notably, an antisense dimer construct was also infectious and transmissible. Importantly, a mutant lacking the AUG start codon of ORF-B lost replication capability, indicating that ORF-B is essential for viral replication. Furthermore, FgAV1 infection suppressed fungal growth and significantly reduced the virulence of *F. graminearum* on wheat. These findings provide novel insights into ambivirus replication and their potential in fungal pathogen biocontrol.

Oral communication 11

Woodchuck and deer hepatitis delta-like agents show distinct replication, innate immune activation and IFN-resistance compared to human hepatitis D virus

G. E. Gnouamozi[1], Z. Zhang[1], S. Seitz[1,3], C. Lauber[3,4], S. Urban[1,2]

[1]Department of Infectious Diseases, Molecular Virology, University Hospital Heidelberg, Heidelberg, Germany; [2]German Center for Infection Research (DZIF), Heidelberg Partner Site, Heidelberg, Germany; [3]German Cancer Research Center (DKFZ), Division of Virus-Associated Carcinogenesis, Heidelberg, Germany; [4]TWINCORE, Centre for Experimental and Clinical Infection Research, Hannover, Germany.
E-mail: GnimahEva.Gnouamozi@med.uni-heidelberg.de

The human hepatitis delta virus (HDV) is a satellite viroid-like RNA virus that relies on hepatitis B virus (HBV) surface proteins (HBsAg) to form infectious virions. For the past 40 years, the evolutionary history of HDV has remained largely unknown. However, recent discoveries have enhanced our understanding of HDV biology. HDV-like agents (DLAs) have been identified across a diverse range of vertebrates and invertebrates, revealing that HDV evolution is more complex than previously thought and is not limited to humans as primary hosts. HDV replication is known to activate interferon (IFN) responses. Both HDV-induced IFNs and exogenous IFN-α and IFN-γ strongly suppress the intracellular spreading pathway of HDV, known as cell division-mediated spread (CDMS). The discovery of DLAs offers the opportunity to study the interplay between delta viruses and IFN responses from an evolutionary perspective.
In this study, we focused on characterizing mammalian DLAs found in the woodchuck (*Marmota monax*, WoDV) and white-tailed deer (*Odocoileus virginianus*, DeDV), in terms of replication, viral spread pathways, and cellular permissiveness. We generated expression constructs encoding 1.1-fold over-length antigenomic RNA of these DLAs and initiated viral replication by transfecting these constructs into both human and non-human hepatic and non-hepatic cell lines. Using a cell division-mediated viral amplification assay, we demonstrated that these DLAs can replicate and propagate via CDMS not only in hepatic tissues, but also without requiring envelopment.
To evaluate the innate immunity induction upon infection and the effect on CDMS, we established a robust infection system for DLAs by packaging their genomes within HBsAg through the trans-complementation of a farnesylated HDV large delta antigen (L-HDAg). The resulting pseudoparticles were used to infect both human and non-human hepatic cells, always in comparison with HDV. As expected, HDV replication triggered IFN activation and consequently restricted CDMS. In contrast, infection with WoDV and DeDV induced a weaker IFN response, and CDMS of these agents remained efficient. Most importantly, the CDMS of WoDV and DeDV was not significantly inhibited by treatment with exogenous IFNs. Thus, these agents not only evade strong IFN activation but also display resistance to IFN-mediated suppression.
Overall, our findings show that DLAs may employ distinct replication, assembly, and immune evasion strategies compared to HDV. These insights deepen our understanding of the molecular biology, evolution, and host interactions of this unique group of viruses, laying the groundwork for future research into immune evasion and viral propagation mechanisms.

Oral communication 12

Establishing a reverse genetic system for a viroid-like element from *Trichoderma spirale*

C. Formiglia[1], M. Forgia[1], B. Navarro[3], O. Rueda[6], F. Di Serio[1], N. Serale[3], S. Oufensou[4], V. Balmas[4], Q. Migheli[4], E. Gobbi[5], N. Miotti[2], F. Bono[5], M. de la Peña[6], M. Turina[2]

[1]Institute for Sustainable Plant Protection, National Research Council of Italy, Strada delle Cacce, 73, 10135 Torino, Italy; [2]Institute for Sustainable Plant Protection, National Research Council of Italy, Viale Europa 11, 25123 Brescia, Italy; [3]Institute for Sustainable Plant Protection, National Research Council of Italy, Via Amendola 165/A, 70126 Bari, Italy; [4]Department of Agricultural Sciences and NRD—Desertification Research Center, University of Sassari, Viale Italia 39a, 07100 Sassari, Italy; [5]Dipartimento di Medicina Molecolare Traslazionale, Università degli Studi di Brescia, Piazza del Mercato, 15, 25121 Brescia, Italy; [6]Instituto de Biología Molecular y Celular de Plantas, Universidad Politécnica de Valencia-CSIC, Valencia, Spain.
E-mail: cristinaformiglia@cnr.it

Viroids are small, single-stranded circular RNAs with high self-complementarity. They lack protein-coding capacity, and some contain paired self-cleaving catalytic RNAs, or ribozymes. Viroid-like RNAs have similar features and are representative of the ribozycirculome, a novel layer of biodiversity recently characterized in fungi. In this study, we assembled an infectious cDNA clone to characterize one of these elements using a reverse genetics approach.
Trichoderma spirale viroid-like RNA 1 (TsvlRNA1) encodes a ribozyme in the plus strand, and was identified in a *Trichoderma spirale* isolate from Sardinia. Northern blot analysis revealed linear and circular forms of both plus and minus polarities *in vivo*. While the self-cleaving activity of the ribozyme in the plus strand was demonstrated *in vitro* and *in vivo*, the specific cleavage on the minus strand remains unidentified.
To establish a reverse genetic system, putative infectious cDNA clones of TsvlRNA1 were generated. Plasmids containing monomers or dimers of the genomic sequence under the transcription of a fungal promoter were transformed into *T. atrobrunneum* and *T. spirale* protoplasts. Transgene integration was confirmed by Real Time-qPCR, and RNA accumulation of TsvlRNA1 was observed in colonies transformed with the dimeric genomic sequence (DIM) but not in those transformed with the monomer (MON). Minus strand-specific RT-qPCR further confirmed true viroid-like replication in the TsvlRNA1 DIM transformed *T. atrobrunneum* and *T. spirale*. This was further supported by the successful horizontal transfer of TsvlRNA1 through direct contact between transformed colonies and recipient isolates belonging to different species of *Trichoderma* therefore demonstrating infectivity through anastomosis.
Additionally, protoplasts of *T. atrobrunneum* were transformed with plasmids containing various genomic deletions, including the AUG of an ORF encoded by TsvlRNA1. Deletions of the whole region coding for the ribozyme abolished completely replication, while a dimer with AUG deleted in both monomers can still replicate and can be transmitted by anastomosis. Interestingly, certain mutations accumulate in the progeny within the region where the AUG codon typically folds into the secondary structure, suggesting that TsvlRNA1 tends to evolve towards a more stable conformation. Additional infectious mutant clones were generated, each carrying point mutations predicted to affect the catalytic activity of the ribozyme. No replication was observed in these cases, demonstrating that an intact and active ribozyme is essential for TsvlRNA1 replication.
This study confirms the viroid-like nature of TsvlRNA1 and deepens our understanding of these infectious elements in fungi, while raising new questions about vegetative compatibility groups in the *Trichoderma* genus.

Oral communication 13

Reservoirs of ancestral Deltaviruses found in water molds

M.J. López-Galiano[1], M. Forgia[2], O. Rueda[1], L. Botella[3], Z. Pasterny[1], A. Babaian[4], M. Turina[5], M. de la Peña[1]

[1]Instituto de Biología Molecular y Celular de Plantas, UPV-CSIC, Valencia, Spain; [2]Institute for Sustainable Plant Protection, National Research Council of Italy, Torino, Italy; [3]Department of Forest Protection and Wildlife Management, Faculty of Forestry and Wood Technology, Mendel University in Brno, Brno, Czech Republic; [4]Department of Molecular Genetics, University of Toronto, Toronto, Canada; [5]Institute for Sustainable Plant Protection, National Research Council of Italy, Brescia, Italy.
E-mail: massimo.turina@cnr.it

The unique human hepatitis delta virus (HDV), also known as hepatitis D agent, is the smallest known virus composed of a ~1,700 nt circular RNA with a rod-like secondary structure. It encodes a small protein (delta antigen) and self-cleaving ribozymes in both genomic polarities (delta ribozymes). Historically regarded as the sole member of its class, recent data have identified novel deltaviruses in diverse metazoans, as well as widespread in environmental metatranscriptomes where the virus is expected to be replicated by unknown hosts. Following high-throughput computational approaches, we uncovered novel and intriguing examples of deltavirus genomes in vertebrate (mammals, lizards and fish), invertebrate (sandfly, deep-sea mussel and termites), and environmental metatranscriptomes. Moreover, minimal deltavirus-like genomes of around 1,200 nt of circular RNA harbouring divergent delta antigens and delta ribozymes were detected in transcriptomic data from two species of oomycetes (water molds). Molecular validation in the causal agent of the grapevine downy mildew, *Plasmopara viticola*, confirmed the pervasive presence of replicative circular RNAs of both polarities of the agent. Altogether, our data points to the water molds, and likely other fungus-like organisms, as potential reservoirs for deltavirus-like agents in metazoans, highlighting the ecological and evolutionary complexity of these minimal replicators.

Oral communication 14

Identification and molecular characterization of viroid-like RNAs with coding capacity: zetaviruses and betaviruses

N. Serale[1], M. Chiumenti[1], P. Mussano[2,3], S. Rotunno[2], F. Venice[3], G. Della Rocca[4], M. Turina[2], A. Mello[5], L. Miozzi[2], F. Di Serio[2], B. Navarro[1]

[1]Institute for Sustainable Plant Protection (IPSP) - Bari Unit, Consiglio Nazionale delle Ricerche (CNR), Bari, Italy; [2]IPSP - Turin headquarter, CNR, Turin, Italy; [3]Department of Life Sciences and Systems Biology, University of Turin, Turin, Italy; [4]IPSP - Sesto Fiorentino Unit, CNR, Sesto Fiorentino, Italy; [5]IPSP - Turin Unit, CNR, Turin, Italy
E-mail: nadiaserale@cnr.it

Soil microbial communities, which live in symbiosis with plants, play a key role in stress resistance, including defense against pathogens. Previous studies on viral and fungal communities associated to soils from a chestnut orchard affected by ink disease, which is caused by the soilborne oomycete *Phytophthora cambivora*, generated metatranscriptomic data that were here analyzed to assess viroid-like RNAs (vdlRNAs) biodiversity through homology-independent analyses (Infernal and vdsearch). Among the vdlRNAs detected, one of them (called soil1090_ZV) was found in two soil samples from healthy chestnut trees and exhibited the typical characteristics of zetaviruses: the genome contains one ORF in each polarity strand encompassing the full-length RNA, thus potentially producing endless tandem-repeat proteins. vdlRNA soil1090_ZV has a circular genome of 600 nucleotides, folds into a rod-like structure, contains hammerhead ribozymes in both strands and harbours sense and antisense ORFs that could encode 200-amino-acid tandem-repeat proteins. In addition, in soil samples from diseased chestnut trees, two novel vdlRNAs (βv786 and βv1793) with new characteristics were identified. Despite 56% nucleotide identity with each other, the two vdlRNAs share the same structural characteristics: a circular RNA genome (664 and 685 nucleotide long) adopting a rod-like secondary structure, containing paired hammerhead ribozymes and two ORFs encompassing the full-length vdlRNA in both polarity strands. The major difference between these new vdlRNAs and zetaviruses is that, in the former, the ORFs do not code for putative tandem-repeat proteins, but have a stop codon located a few nucleotides after the start of the second monomer, resulting in a ORF of about 10 nt longer than the entire genomic and antigenomic RNA. We called this new type of vdlRNAs betaviruses (βv786 and βv1793). Using RT-PCR, circular forms of both polarity strands of the zetavirus soil1090_ZV and the two betaviruses βv786 and βv1793 were detected in RNA preparations from soil, thus providing the first conclusive evidence of their existence as circular RNAs. Moreover, for all these vdlRNAs, the absence of DNA counterpart was confirmed and the self-cleaving ribozymatic activities and the specific sites of self-cleavage were established by *in vitro* transcription and 5'RACE. Finally, we searched for other betavirus-like in metatranscriptomic databases, and we identified nine putative betaviruses associated with environmental samples from wetlands of different geographic areas, thus supporting the notion that betaviruses are a new group of vdlRNAs likely present in several ecological niches. Alphafold structure prediction of the putative betavirus-encoded proteins showed a certain structural conservation in the predicted folding.

Oral communication 15

Salicylic acid modulates volatile organic compound profiles during CEVd infection in tomato plants

M. Balanzá, F. Vázquez-Prol, I. Rodrigo , J.M. Bellés, F. Vera-Sirera, M.P. López-Gresa, P. Lisón

Instituto de Biología Molecular y Celular de Plantas, Universitat Politècnica de València (UPV)-Consejo Superior de Investigaciones Científicas (CSIC), Camino de Vera s/n, 46022 Valencia, Spain.
E-mail: marbaga1@alumni.upv.es; plison@imbcp.upv.es;

Background: Citrus exocortis viroid (CEVd) is a non-coding RNA pathogen capable of infecting a wide range of plant species, despite its lack of protein-coding ability. Viroid infections induce significant alterations in various physiological and biochemical processes, particularly impacting plant metabolism. This study shows the metabolic changes upon viroid infection in tomato plants (*Solanum lycopersicum* var. 'MoneyMaker') exhibiting altered levels of salicylic acid (SA), a key signal molecule involved in the plant defence against this pathogen. Methods: Transgenic *RNAi_S5H* lines, which have the salicylic acid 5-hydroxylase gene silenced to promote SA accumulation, and *NahG* lines, which overexpress a salicylate hydroxylase to degrade SA into catechol and prevent its accumulation, were used to establish different SA levels in plants, resulting in varying degrees of resistance to viroid infection. The analysis was performed by using gas chromatography–mass spectrometry (GC-MS) to explore the role of volatile organic compounds (VOCs) in plant immunity against this pathogen. Results: Our results revealed distinct volatile profiles associated with plant immunity, where *RNAi_S5H*-resistant plants showed significantly enhanced production of monoterpenoids upon viroid infection. Moreover, viroid-susceptible *NahG* plants emitted a broad range of VOCs, whilst viroid-tolerant *RNAi_S5H* plants exhibited less variation in VOC emission. Conclusions: This study demonstrates that SA levels significantly influence metabolic responses and immunity in tomato plants infected by CEVd. The identification of differential emitted VOCs upon CEVd infection could allow the development of biomarkers for disease or strategies for disease control.

Oral communication 16

Antiviral immunity and suppression by RNA viruses

C. Huang[1,2], A. Sede[1], L. Elvira-Gonzalez[1], Y. Yan[3,4], M. Rodriguez[1,3,4], J. Mutterer[1], E. Boutant[1,5], L. Shan[3,4], M. Heinlein[1]

[1]Institut de Biologie Moléculaire des Plantes, CNRS, Université de Strasbourg, 67000, Strasbourg, France; [2]Beijing Academy of Agriculture and Forestry Sciences, Beijing, China; [3]Department of Molecular, Cellular, and Developmental Biology (MCDB), University of Michigan, Ann Arbor, Michigan, USA; [4]Department of Biochemistry and Biophysics, Texas A&M University, College Station, Texas, USA; [5]Faculté de Pharmacie, Université de Strasbourg, Strasbourg, France.
E-mail: manfred.heinlein@ibmp-cnrs.unistra.fr

In addition to its functions in antiviral RNA silencing, dsRNA elicits pattern-triggered immunity (PTI), contributing to plant resistance against virus infections (Niehl et al., 2016). However, the mode-of-action and signalling pathway of dsRNA-induced defence remain poorly characterised. In a study using multicolor in vivo imaging, analysis of GFP mobility, callose staining, and plasmodesmal marker lines in *Arabidopsis thaliana* and *Nicotiana benthamiana*, we showed that dsRNA-induced PTI restricts the progression of virus infection by triggering callose deposition at plasmodesmata, thereby limiting the macromolecular transport through these cell-to-cell communication channels (Huang et al., 2023; Heinlein, 2025). The dsRNA-induced signalling leading to callose deposition at plasmodesmata involves plasma membrane-resident SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE 1, the BOTRYTIS INDUCED KINASE1/AVRPPHB SUSCEPTIBLE1-LIKE KINASE1 kinase module, PLASMODESMATA-LOCATED PROTEINs 1/2/3, as well as CALMODULIN-LIKE 41 and Ca2+ signals. Interestingly, unlike the well-known PTI elicitor flg22, dsRNA does not trigger a detectable reactive oxygen species (ROS) burst. Likely as a counter strategy, viral movement proteins from different viruses suppress the dsRNA-induced host response to achieve infection. Our data support a model by which plant immune signaling constrains virus movement by inducing callose deposition at plasmodesmata and how viruses counteract this layer of immunity. The results challenge the classical view that viral movement proteins "open" plasmodesmata. Rather, these proteins act as viral effectors that prevent plasmodesmata closure by interfering with a dsRNA-induced immunity response.

References:

Niehl A, Wyrsch I, Boller T, Heinlein M (2016) Double-stranded RNAs induce a pattern-triggered immune signaling pathway in plants. *New Phytologist* 211, 1008-19

Huang C, Sede AR, Elvira-Gonzales L, Yan Y, Rodiguez M, Mutterer J, Boutant E, Shan L, Heinlein M (2023) dsRNA-induced immunity targets plasmodesmata and is suppressed by viral movement proteins. *The Plant Cell* 35, 3845-3869.

Heinlein M (2025) Antiviral double-stranded RNA sensing immunity in plants. *Annual Review of Virology*, in press.

Oral communication 17

Exploring extracellular vesicles (EVs) during viral and viroid infections

K. Katsarou[1,2], K. Kalantidis[1,2]

[1]Department of Biology, University of Crete, Voutes University Campus, Heraklion, Crete, Greece; [2]Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, Heraklion, Crete, Greece.
E-mail: Katsarou@imbb.forth.gr

Communication between cells, either of the same or different organisms, is crucial for the function of multicellular organisms. Extracellular vesicles (EVs) have emerged as a sophisticated pathway to achieve precise and efficient transport of molecules between cells and organisms. EVs are classified into different types based on their size, origin, and biogenesis. Their composition reflects the characteristics of the cell that produces them, as well as the physiological or pathological condition of the organism.
EVs have been implicated in viral pathogenesis. Studies focusing mostly on viruses infecting mammalian organisms suggest that EVs play an important role in viral transmission within or between hosts, in regulation of gene expression often targeting antiviral pathways, and even acting as a decoy scavenging virions and limiting virus entry, or binding secreted host factors in the environment (such as antibodies in the case of animal viruses).
However, to date there is limited information about the role of EVs in plant virology. In this study, we developed protocols to purify EVs from both *Nicotiana benthamiana* and *Solanum lycopersicum* plants, and used techniques like TEM and NTA to assess their quality. Then, we investigated if viruses (e.g TuMV, CMV, TRV etc) and viroids (PSTVd) induce EVs production during infection. Finally, we are investigating the content present in these EVs. This analysis will pave the way for understanding the biological cycle of these pathogens and explore the role of EVs during viral and viroid infections.

Oral communication 18

Small RNA-mediated host regulation in citrus bark cracking viroid-infected hop plants

M. H. Hagemann[1], S. Jagani[1], U. Born[1], J. Jakše[2]

[1]University of Hohenheim, Institute of Phytomedicine, 70599 Stuttgart, Germany; [2]University of Ljubljana, Biotechnical Faculty, SI-1000 Ljubljana, Slovenia.
E-mail: michael.hagemann@uni-hohenheim.de

The viroid infection is typically associated with changes in transcriptional regulation of its host plant, including alterations in the small RNA profile, which may contribute to viroid-induced pathogenesis. To extend knowledge in this field, we present preliminary small RNA data from *Humulus lupulus* infected with citrus bark cracking viroid (CBCVd) and hop latent viroid (HLVd), compared to plants infected only with HLVd, as commonly observed in commercial hop production.
Focusing on candidate small RNA-mediated host regulation under controlled environmental conditions, we aimed to link differentially expressed miRNAs to putative gene functions via target prediction and qPCR validation.
Controlled greenhouse trials demonstrated that viroid-associated stunting was enhanced under elevated temperatures. Several plant microRNAs exhibited altered expression in response to CBCVd infection, targeting pathways related to hormonal regulation, protein folding, stress signaling, and sulfur metabolism. Differential miRNA-mRNA regulation was confirmed for selected targets. Additionally, viroid-derived small RNAs (vd-sRNAs) were identified, showing strong accumulation at a hotspot within the central conserved region (CCR), shared between CBCVd and HLVd. This hotspot may contribute to early stress signaling and potentially to the synergistic symptom development observed in co-infections.
These results provide initial insights into early regulatory events during viroid-host interactions and highlight the need for integrative analyses of combinatorial small RNA activities and their role in viroid-induced stress syndromes.

Oral communication 19

Role of small RNAs in disease symptom recovery

L. Elvira-González[1], C. Matteoli[1], C. Himber[1], T. Blevins[1], V. Schurdi-Levraud[2], M. Heinlein[1]

[1]Institut de Biologie Moléculaire des Plantes, CNRS, Université de Strasbourg, 67000, Strasbourg, France; [2]Université de Bordeaux, INRAE, Biologie du Fruit et Pathologie, UMR 1332, 33140 Villenave d'Ornon, France.
E-mail: laura.elvira@ibmp-cnrs.unistra.fr

Plants in natural ecosystems exhibit inherent tolerance to viral infections, enabling viral replication and systemic movement while maintaining overall fitness and reproductive success. In *Arabidopsis thaliana*, tolerance to viruses depends on virus species and also varies across ecotypes, providing a unique model to investigate this phenomenon. For instance, the Col-0 ecotype tolerates infection by tobacco mosaic virus (TMV) whereas it shows strong disease symptoms upon infection with oilseed rape mosaic virus (ORMV). Interestingly, ORMV-infected Col-0 plants can recover from disease symptoms. Recovery occurs despite of continued viral replication thus indicating a transition into a state of "induced tolerance".
Induced tolerance to ORMV depends on key components of post-transcriptional and transcriptional gene silencing (PTGS and TGS) involved in small RNA (sRNA) synthesis, such as RNA-dependent RNA polymerase 2 and 6 (RDR2/6), DICER-LIKE proteins 2 and 4 (DCL2/4), and RNA polymerase IV (Pol IV). Consistently, disease symptom recovery is associated with the accumulation of virus-derived siRNAs (vsiRNAs) and host-derived siRNAs, such as virus-activated siRNAs (vasiRNAs) and Pol IV-dependent siRNAs. These siRNAs, generated in symptomatic tissues, likely move systemically into newly emerging leaves and compete with the miRNA sequestering activity of the viral suppressor of RNA silencing (VSR), resulting in symptom recovery (Kørner et al., 2018; Elvira-González et al., 2025).
This study highlights the intricate interplay between viral VSRs and host siRNAs in modulating disease symptoms versus tolerance. Further investigation is required to understand the tissue-specific dynamics of siRNA production and movement, as well as the modulation of these processes by different viruses and environmental factors. Overall, these studies pave the way for new insights into the mechanisms underlying viral pathogenesis and host-virus interactions in plants.

References:

Kørner CJ, Pitzalis N, Peña EJ, Erhardt M, Vazquez F, Heinlein M. 2018. Crosstalk between PTGS and TGS pathways in natural antiviral immunity and disease recovery. *Nature Plants* 4, 157-164.

Elvira-González L, Blevins T, Heinlein M (2025) Small RNA mobility and plant virus diseases. *Journal of Experimental Botany*, eraf226 (online ahead of print).

Oral communication 20

Decoding hop–viroid interactions: a transcriptomic approach to CBCVd resistance

H. Volk[1], S. Radišek[2], A. Čerenak[2], J. Jakše[1]

[1]Biotechnical Faculty, Department of Agronomy, University of Ljubljana, Jamnikarjeva 101, 1000 Ljubljana, Slovenia [2] Slovenian Institute of Hop Research and Brewing, Cesta Žalskega tabora 2, 3310 Žalec, Slovenia. E-mail: helena.volk@bf.uni-lj.si

The unexpected emergence of Citrus bark cracking viroid (CBCVd; *Cocadviroid rimocitri*) as a major threat to hop cultivation has alarmed producers worldwide, with Slovenia and Germany among the most affected regions. Originally considered a minor, economically insignificant pathogen confined to citrus, CBCVd was unexpectedly identified in hops in Slovenia in 2014. Its transmission to a non-citrus host, coupled with devastating effects such as plant death within five years, signalled a critical shift in the viroid's epidemiology. The initial introduction is suspected to have occurred via infected citrus waste, with rapid spread facilitated by mechanical transmission and vegetative propagation methods common in hop production.

Field observations in Slovenia have since highlighted varying degrees of CBCVd susceptibility across hop cultivars, identifying Styrian Wolf and Styrian Cardinal as promising candidates for resistance and tolerance, respectively. In an effort to further understand and mitigate the threat, a comprehensive study was conducted using tissue culture-based artificial inoculation, grafting experiments, and advanced molecular techniques.

Controlled inoculation of hop cultivars in tissue culture eliminated confounding pre-existing infections and ensured uniform viroid exposure. Disease progression was assessed over a seven-week period using disease severity indices and RT-PCR confirmation. Complementary grafting trials involved scions from infected Celeia plants grafted onto rootstocks of Styrian Wolf (presumably resistant cultivar), Styrian Cardinal (presumably tolerant cultivar), and Celeia (susceptible cultivar) to assess transmission dynamics and resistance traits under secured conditions.

To better understand the molecular basis of resistance, transcriptomic profiling was performed on infected and healthy plant samples. RNA sequencing using the MGI-T7 platform yielded 20.3–40.3 million reads per sample. Bioinformatics analysis revealed differentially expressed genes (DEGs) distinguishing resistant from susceptible cultivars.

Collectively, these findings provide a critical foundation for breeding CBCVd-resistant hop cultivars and offer new insights into plant-viroid interactions. The integration of phenotypic evaluations, grafting trials, and transcriptomics represents a comprehensive approach toward sustainable viroid management in hops.

Oral communication 21

Detection and characterization of viroid replication intermediates in hop using nanopore sequencing

A. Sečnik[1], H. Volk[1], N. Štajner[1], J. Jakše[1]

[1]University of Ljubljana, Biotechnical Faculty, Agronomy Department, Jamnikarjeva 101, 1000 Ljubljana, Slovenia.
E-mail: jernej. jakse@bf.uni-lj.si

Nuclear viroids are small non-coding RNA pathogens that replicate in the host nucleus via an asymmetric rolling-circle mechanism, producing multimeric intermediates of both positive (+) and negative (-) polarity. Classical detection approaches for detecting these replication intermediates, such as gel electrophoresis and blotting, are limited in resolution and throughput. In this study, we tested and employed Oxford Nanopore Technologies (ONT) direct RNA sequencing to qualitatively and quantitatively investigate viroid replication intermediates in hop (*Humulus lupulus*) infected with Citrus bark cracking viroid (*Cocadoviroid rimocitri*, CBCVd), hop latent viroid (*Cocadviroid latenshumuli*, HLVd), and hop stunt viroid (*Hostuviroid impedihunuli*, HSVd). Proof-of-concept sequencing of an *in-vitro*-transcribed CBCVd RNA dimer generated ~1.5 × 10^6 reads, confirming that ONT can recover full-length viroid molecules. Biological samples were then processed with four library strategies—native total RNA, rRNA-depleted RNA, and viroid-enriched RNA obtained with either biotinylated DNA probes or MagIC capture beads. Different flow cell formats (MinION vs. Flongle) were also tested. Although ONT has lower read accuracy compared to short-read technologies, it offers unique advantages in capturing full-length viroid RNA and their multimeric forms. Across different biological libraries, we produced > 4.5 × 10^6 reads. Although viroid reads represented only a minority of the total reads, capture-based enrichment improved recovery. The MagIC/Flongle libraries yielded up to a 35-fold increase relative to unenriched controls. Comparative analysis revealed notable differences in the abundance and composition of replication intermediates across viroid species. HLVd showed the highest proportion of multimeric intermediates, followed by CBCVd and HSVd. In CBCVd-infected samples, (+) monomers were most abundant, followed by (-) monomers. For HLVd, the most abundant forms were also (+) monomers, with significant fractions of dimers and trimers. The presence of longer intermediates supports the rolling-circle replication model, while the relative abundance of (+) and (-) strands may indicate viroid-specific replication dynamics. Furthermore, we attempt to predict putative transcription sites by analyzing read start distributions in the sequencing data, which may reveal potential initiation hotspots. Our work demonstrates the applicability of ONT RNA sequencing in viroid biology and provides novel insights into the diversity of replication intermediates. Optimized protocols could also offer a valuable foundation for developing next-generation diagnostics and investigating host-viroid interactions.

Oral communication 22

Exploring RNA modifications in viroids: detection of N⁶-methyladenosine (m^6A) in citrus exocortis viroid

M. Chiumenti[1], P. Vopalensky[2], A. Škríba[2], L. Ďuričeková[2,3], A. Šimonová[2], O. Lukšan[2], F. Di Serio[1], B. Navarro[1], H. Cahova[2]

[1]Institute for Sustainable Plant Protection (IPSP), National Research Council of Italy (CNR), Italy; [2]Institute of Organic Chemistry and Biochemistry of the CAS, Prague, Czechia, [3]Faculty of Science, Department of Cell Biology, Charles University, Prague, Czechia.
E-mail: michela.chiumenti@cnr.it

RNA plays a central role in all cellular processes, with its functional information encoded across three layers: sequence, structure and chemical modifications. While RNA modifications are well-characterized in abundant RNA species such as rRNA and tRNA, their roles in less represented RNA types—like mRNA, regulatory RNAs, and viral RNAs—remain less understood. In this context, infectious non-coding agents, such as viroids, represent an interesting opportunity to uncover RNA modifications and explore their biological roles. Viroids are non-coding small circular RNAs. In the absence of protein coding capacity, their infectivity depends on host enzymes or intrinsic structural features. These structures likely mimic host nucleic acid motifs, as such, viroids serve as valuable models to investigate RNA structure-function relationships. Whether RNA modifications exist and could play a role in viroid biology remains still unknown. So far, attempts to identify RNA modifications in viroids have employed the bisulfite sequencing, which did not reveal any 5-methylcytosine (m^5C) in these infectious RNAs. In this study, we examined the presence of 15 different RNA modifications in purified circular RNA forms of two viroids: avocado sunblotch viroid (ASBVd, *Avsunviroidae*) and citrus exocortis viroid (CEVd, *Pospiviroidae*), representative members of viroids replicating in chloroplasts and nucleus, respectively, by liquid chromatography–mass spectrometry (LC–MS). Quantitative analysis of the LC-MS data revealed the presence of approximately one N⁶-methyladenosine (m^6A) modification per molecule of CEVd, with no other modifications detected. ASBVd showed no detectable modifications. To identify the position of m^6A in the CEVd RNA, we employed Oxford Nanopore Technology (ONT) direct sequencing of purified circular CEVd from infected tissues, in combination with several m^6A prediction algorithms. These algorithms identified three candidate positions. Validation, using a single base elongation- and ligation-based qPCR amplification method (SELECT), confirmed the presence of m^6A at two of the three candidates, A353 and A360, which are highly conserved among CEVd variants. Further analysis showed that the GGNACC motif surrounding CEVd A353 is conserved in the terminal left region of the rod-like structure of all the species of the genus *Pospiviroid,* to which CEVd belongs. This region includes binding sites for RNA polymerase II and TFIIIA-7ZF, proteins involved in nuclear viroid replication, as demonstrated in potato spindle tuber viroid*,* the representative member of this genus. These findings suggest a potential role for m^6A methylation in viroid replication.

Oral communication 23

Harnessing citrus viroids as natural dwarfing agents for economic optimization in high-density citrus orchards

I. Lavagi-Craddock[1], V. Lavagi[2], S. Bodaghi[1], G. Villalba-Salazar[1], S. Hajeri[3], J. Kaplan[4], M. Gómez[2], A. El-Kereamy[1], G. Vidalakis[1]

[1]University of California, Riverside, CA 92521, U.S.A.; [2]Cornell University, Ithaca, NY 14853, USA; [3]Alliance of Pest Control Districts, Tulare, CA 93274, USA; [4]California State University, Sacramento, CA 95819, U.S.A.
E-mail: vidalg@ucr.edu

Citrus viroids have been studied not as pathogens but rather as transmissible small nuclear RNAs (TsnRNAs) acting as genetic elements regulating tree growth for the production of high-density plantings of commercial citrus, allowing for improved orchard management and cost efficiencies. An experimental plot of TsnRNA-treated navel orange trees planted in 1998 demonstrated the feasibility and practical utility of TsnRNA-induced dwarfing. Through a cost-benefit analysis conducted from 2018 to 2023, we evaluated the impact of these dwarf trees on labor savings, yield revenue, and overall profitability. Results show that the smaller canopy facilitates easier harvesting and pest inspection, lowering labor costs while maintaining fruit quality and competitive yields. Higher planting densities - nearly double the 1998 standard - led to optimized land use and potentially increased fruit production per acre. In a 20-year simulation, groves planted with TsnRNA dwarfed trees supported 200 trees per acre, compared to 100 standard trees. Accounting for both higher yields and reduced harvest labor, an acre of dwarf trees was estimated to generate up to $292 more in net annual returns than an acre of standard trees-driven by approximately $244 in added revenue (an 18% increase) and $48 in labor savings (an 18% reduction in harvest labor costs). This strategy provides growers with a tool to mitigate economic losses associated with diseases or tree eradication programs, land scarcity, and labor shortages. For example, in huanglongbing (HLB)-affected groves, where tree mortality and declining productivity increase financial risk, the economic advantage becomes even more pronounced. Because each dwarf tree contributes a smaller share of total production, losses are less disruptive and easier to replace. Net returns in HLB-impacted blocks may exceed $400 per acre annually. When adjusted for tree replacement and maintenance costs, economic modeling still favored the TsnRNA trees, especially under tree loss or eradication conditions. The trees treated with TsnRNA-IIIb (citrus dwarfing viroid) had 41–45% of the canopy volume of non-treated controls (NTCs) and produced 49–66% of the fruit yield at two different planting densities, respectively. TsnRNA-IIa (hop stunt viroid) trees retained 82% canopy size and yielded 84% of NTCs. As such, corresponding labor time reductions for harvesting, skirting, and pest inspections ranged from 44–58%, 44–56%, and 36–42% respectively for TsnRNA-IIIb-treated trees. These patterns were replicated in a 29-year-old commercial orchard, where TsnRNA-IIIb-treated ‘Cara Cara’ navel trees retained just 40% of the canopy volume and reduced pest inspection time by 36%, while remaining productive and easy to manage. These findings highlight viroid-induced dwarfism as a scalable and economically viable alternative for modern citrus management, underscore the translational potential of viroid research beyond plant pathology, and support regulatory and industry discussions on the intentional deployment of economically and operationally beneficial viroids for commercial citrus production.

Oral communication 24

Optimization of the viroid-double-self-splicing-intron system to produce recombinant RNA in *Escherichia coli*

D. Palpacelli[1], M. Spada[2], S. Pecchia[2], J.A. Daròs[1]

[1]Instituto de Biología Molecular y Celular de Plantas, CSIC-Universitat Politècnica de València, Avenida de los Naranjos s/n, 46022 Valencia, Spain; [2]Department of Agriculture Food and Environment, University of Pisa, Via del Borghetto 80, 56124 Pisa, Italy.
E-mail: dpalpac@doctor.upv.es; jadaros@ibmcp.upv.es

Co-expression in *Escherichia coli* of a longer-than-unit precursor of eggplant latent viroid (ELVd; *Avsunviroidae*; *Elaviroid*), along with the chloroplastic isoform of eggplant tRNA ligase, results in an unexpected massive accumulation of the viroid circular form. Especially, because no detection of viroid replication intermediates in *E. coli* supports lack of viroid replication in these bacterial cells. Based on this observation, we developed a system to produce recombinant RNA in *E. coli* in which the ELVd molecule acts as a scaffold on which an RNA of interest is grafted. This way, always with the co-expression of the eggplant tRNA ligase, large amounts of chimeric recombinant RNAs composed of the ELVd scaffold and the RNAs of interest can be produced in *E coli*. In a second version of this system, we introduced two type-I self-splicing introns, specifically *Tetrahymena thermophila* 26S rRNA intron, to produce recombinant circular double-stranded RNA (dsRNA) in which the viroid scaffold is spliced out. In this new system, the first self-splicing intron separates the two strands of the recombinant dsRNA, stabilizing the plasmid construct, while the second self-splicing intron, which is inserted flanking the RNA of interest in a permuted manner, acts on the viroid scaffold removal, yielding a recombinant circular RNA product. We have recently shown that this kind of circular dsRNA molecules can be used in crop protection inducing RNA interference (RNAi) in plant pests and pathogens, such as the Western corn rootworm *Diabrotica virgifera*, the medfly *Ceratitis capitata* or the South African mealybug that has recently invaded citrus plantations in the Mediterranean basin, *Delottococcus aberiae*. Work on the polyphagous grey mold *Botrytis cinerea* is also in progress.
While the extended double-stranded nature of the circular recombinant dsRNAs must contribute to the stability of the molecule, the two 20-nt single-stranded loops that lock the molecules at both ends are perceived as potential elements of instability. These loops originate from the 10-nt exon fragments that flank both type-I self-splicing introns in the recombinant system. In this work, we aimed to reduce the size of these two terminal loops without compromising accumulation of the recombinant circular dsRNA. For this purpose, we built a series of constructs for *in vitro* transcription in which two opposite strands of a *B. cinerea* MAP kinase (*BcBmp1*) gene fragment were separated with different versions of the *T. thermophila* 26S rRNA self-splicing type-I intron with different deletions of the exon fragments at both sides. RNA from the different constructs was produced *in vitro* using phage T7 RNA polymerase. The products of the *in vitro* transcription reaction were separated by polyacrylamide gel electrophoresis and quantified after ethidium bromide staining of the gel. The ratio among the spliced and non-spliced forms of the transcripts informed about the effect of the different exon deletions on self-splicing efficiency. Results indicated that substantial deletions of the exon fragments is possible with no effect on self-splicing.
We are currently transferring this finding to the *E. coli* viroid-double-self-splicing-intron system to produce new recombinant circular dsRNA versions with minimal terminal loops.

Oral communication 25

Synthetic circular RNAs and their potential use in plant protection

M. Murr[1], A. Koch[1], T. Schlemmer[1]

[1]Department of Plant Cell Biology, Biochemistry and Biotechnology, University of Regensburg, 93053 Regensburg, Germany.
E-mail: Timo.Schlemmer@ur.de

Synthetic circular RNAs are covalently closed, single-stranded RNAs that lack open 5' and 3' ends leading to increased exonuclease resistance. As functions of synthetic circular RNAs rely on their sequence, their potential use strongly coupled to the invested sequence. Therefore, multiple functions and biotechnological usages are possible. Thus, synthetic circular RNAs may induce target gene silencing, work as (micro) RNA or protein sponges or coding for proteins when carrying an open reading frame downstream of an internal ribosomal entry site.
Here we investigate the use of small circular antisense RNAs to induce target gene silencing of essential genes in the maize infecting smut fungus *Ustilago maydis*. Therefore, we are developing a time and cost-effective circular RNA synthesis plattform to test this novel type of RNAs and their functions in a plant protective context. So far, we saw antisense circular RNA stability over one week post spray application onto maize seedlings and flowering maize plants in directly treated and non-treated leaves. Based on these findings, we speculate circular RNA uptake into maize plants and translocation within the plants towards non-treated leaves. Furthermore, we designed circular antisense RNAs against *Ustilago maydis* and tested them on their target sequence binding ability *in vitro* and sprayed them onto maize seedling before infection with *Ustilago maydis*. The majority of the tested circular antisense RNAs showed RNA-RNA interaction and a decrease in infection rates, indicating successful inhibition of the fungal pathogen in our experimental setup.

Poster 1

Symptom induction from non-infectious forms of citrus exocortis viroid in *Nicotiana benthamiana*

V. Aragonés[1], M. Eiras[1,2], J.A. Daròs[1]

[1]Instituto de Biología Molecular y Celular de Plantas, CSIC-Universitat Politècnica de València, Avenida de los Naranjos s/n, 46022 Valencia, Spain; [2]Lab. Fitovirologia e Fisiopatologia, Centro de Pesquisa de Sanidade Vegetal, Instituto Biológico, CEP 04014-002, São Paulo, SP, Brazil; M.E. supported by FAPESP fellowship (proc.19/20746-3).
E-mail: varagoni@ibmcp.upv.es; marcelo.eiras@sp.gov.br; jadaros@ibmcp.upv.es

Viroids are small, circular, non-coding RNAs typically infectious to higher plants, although recent research is expanding this concept, and viroid-like RNAs that apparently fulfill the viroid concept have been found associated to hosts from other kingdoms, particularly fungi. Viroid infections of higher plants range from devastating, such as the cadang-cadang in coconut palms, to asymptomatic, such as eggplant latent viroid. Interestingly, symptoms of viroid infections do not necessarily correlate with viroid accumulation, with well-known examples of asymptomatic avocado trees that accumulate large amounts of avocado sunblotch viroid. This is in contrast to what usually occurs with plant viruses, in which symptom intensity usually correlates with viral accumulation in infected tissues. How such a small non-coding RNA induces symptoms of infection in the host plant has been a long-standing mystery, until recent reports indicating that viroid-derived small RNAs arising from the host defensive RNA silencing machinery are most probably responsible. Plants, like many other eukaryotes, use RNA silencing pathways to defend from invading nucleic acids. Structured viroid RNAs or double-stranded replication intermediates are processed by Dicer-like enzymes to produce viroid-derived small interfering RNAs (siRNAs) in a process that is amplified by host RNA-dependent RNA polymerases. Viroid-derived siRNAs are finally load by Argonaute proteins to form the RNA-induced silencing complex that targets homologous RNAs. This way, based on homology with host transcripts, accumulation of viroid-derived siRNAs may lead to physiological alterations in the host plants. This mechanism was first demonstrated to explain the yellowing symptoms of the Y strain of cucumber mosaic virus satellite RNA. Later, this mechanism was also proposed to explain symptoms of viroid infections in both families, the calico induced by certain sequence variants of peach latent mosaic viroid (family *Avsunviroidae*) or the tuber deformation induced by potato spindle tuber viroid (family *Pospiviroidae*).
To gain insight into this symptom induction mechanism, we performed experiments in which citrus exocortis viroid (CEVd; *Pospiviroidae*; *Pospiviroid*) forms were expressed in *Nicotiana benthamiana* using a viral vector derived from potato virus X (PVX). Expression of longer-than-unit CEVd forms produced double infections with symptoms more severe than those induced in the PVX single-infected controls. Interestingly, PVX-based expression of CEVd non-infectious forms with deletions in the left terminal end of the molecule also produced more intense symptoms, which were intermediate between those of the PVX single-infected plants and those of the PVX-CEVd double-infected plants. These results suggest that, even in the absence of CEVd infection, production of CEVd RNAs in *N. benthamiana* by means of the PVX vector induces particular symptoms of that are added on top of those induced by the PVX vector alone. This observation is compatible with an RNA silencing mechanism in which viroid-derived siRNAs produced during the replication of the PVX recombinant clone target specific host genes. This observation also raised the intriguing question of why this mechanism of symptom induction is particularly frequent in viroids. Despite having genomes, at least, one order of magnitude larger than those of viroids, this symptom induction mechanism does not seem to be widespread in plant viruses. We hypothesize that evolution may have particularly shaped the small viroid genomes to target specific host genes.

Poster 2

Exploring the role of VIRP1, a bromodomain-containing protein crucial for pospiviroid infectivity

E. Bardani[1,4], K. Katsarou[1], C. Andronis[2], S. Ostendorp[3], J. Kehr[3], K. Kalantidis[1,2]

[1]Department of Biology, University of Crete, Voutes University Campus, Heraklion, Crete, Greece; [2]Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, Heraklion, Crete, Greece; [3]Institute of Plant Science and Microbiology, University of Hamburg, Ohnhorststr. 18, Hamburg, 22609, Germany; [4]Current address: Department of Biology, Swiss Federal Institute of Technology (ETH-Zürich), Zürich, 8092, Switzerland.
E-mail: ebardani@eth.ch

VIRP1 (Viroid binding protein 1) was identified in tomato plants infected by potato spindle tuber viroid (PSTVd) to strongly and specifically interact with the viroid RNA through an atypical RNA-binding domain. VIRP1 is crucial for viroid infectivity, as *VIRP1*-suppressed tobacco plants showed complete resistance to PSTVd mechanical inoculation. VIRP1 localizes in the nucleus and carries a bromodomain, which recognizes acetylated histones, thereby interpreting the "open chromatin" signal to modulation of gene expression. Nevertheless, the endogenous function of VIRP1, as well as its role in the viroid biological cycle remain elusive. Here, we explored the effect of *VIRP1* loss-of-function on the phenotype and gene expression patterns in *Nicotiana benthamiana*, highlighting a link to stress responses. We further show that VIRP1 promotes liquid-liquid phase separation, a property that is affected by the presence of PSTVd RNA. Using a virus-based complementation system, we investigated the effect of different VIRP1 mutations/truncations on PSTVd infectivity. Our results show that VIRP1 nuclear import is important for viroid infectivity, as previously suggested, however PSTVd can enter the nucleus even in the absence of VIRP1. Importantly, mutations in a conserved bromodomain residue severely reduce viroid titers, pinpointing a putative role of VIRP1 in PSTVd subnuclear localization/trafficking.

Poster 3

Double trouble: viral and viroid conspiracies in host cells

K. Katsarou[1,2], Z. Pentheroudaki[2], A. A. Nikoloudi[2], C. Spiridaki[2], N. Papadima-Karanikou[2], K. Kalantidis[1,2]

[1]Department of Biology, University of Crete, Voutes University Campus, Heraklion, Crete, Greece; [2] Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, Heraklion, Crete, Greece.
E-mail: katsarou@imbb.forth.gr

Co-infection, the occurrence of multiple pathogens infecting a single host organism, is a naturally observed phenomenon. In plants, mixed infections involving viruses, fungi, and bacteria constitute a significant area of research. These studies have uncovered key biomolecular pathways and mechanisms underlying host-pathogen interactions, revealing a spectrum of outcomes (synergistic, antagonistic, and neutral relationships) that highlight the complexity of co-infection dynamics. On the contrary, information regarding virus-viroid co-infections remains limited.
Here, we used *Nicotiana benthamiana* as a model system due to its high permissiveness to both viruses and viroids, and tested various viruses like cucumber mosaic virus (CMV), and tomato spotted wilt virus (TSWV), and viroids e.g. potato spindle tuber viroid (PSTVd) during co-infections. Pathogens were inoculated under two conditions: either the virus was inoculated before the viroid or *vice versa*. Using phenotypic assessment and Northern blot assays for the detection and quantification of the desired RNAs, we identified distinct response patterns for each pathogen. These findings reveal that each virus–viroid pairing influences the host in unique ways, underscoring the complexity and diversity of host-pathogen interaction motifs involved in co-infections.

Poster 4

Molecular insights into viroid-induced defense mechanisms in grafted tomato plants

R. Marziale[1], M. Chiumenti[1], R. Spano[1], G. Bubici[1], A. Petrozza[2], F. Cellini[2], B. Navarro[1], F. Di Serio[3]

[1]Institute for Sustainable Plant Protection, National Research Council of Italy CNR- SS Bari, Via Amendola 165/A, 70126, Bari, Italy; [2]Research Centre of the Lucana Agency for Development and Innovation in Agriculture (ALSIA) Metapontum Agrobios, Metaponto, Italy. [3] Institute for Sustainable Plant Protection, National Research Council of Italy CNR- Turin, Strada delle Cacce 73, 10135 Torino, Italy.
E-mail: francesco.diserio@cnr.it

Grafting may enhance the performance and potential resilience against biotic and abiotic stresses in various horticultural crops. While certain tomato-virus combinations have demonstrated increased tolerance to viral infections through grafting, its impact on infections caused by potato spindle tuber viroid (PSTVd; family *Pospiviroidae*)—a severe pathogen responsible for stunting and leaf curling in tomato—remains unclear. Here, the influence of grafting on tomato plant responses to PSTVd infection was investigated using high-throughput imaging phenotyping and transcriptomic analyses. The tomato cultivars Manduria (Ma), known for its tolerance to viral infections, and UC82 (UC), more susceptible were tested. Following mechanical inoculation with PSTVd or mock treatment, morphological parameters were quantitatively assessed across nine time points up to 36 days post-inoculation (dpi) in non-grafted, self-grafted UC (UC/UC), and UC grafted onto Ma (UC/Ma) plants. Principal component analysis (PCA) revealed that grafting has a global effect on the phenotype of PSTVd-infected plants. Transcriptomic profiling at 15 dpi indicated a higher and more diverse number of differentially expressed genes in ungrafted plants (Ma, UC) than in grafted counterparts (UC/UC, UC/Ma), with gene ontology analyses indicating that plants response to grafting and to PSTVd largely overlap. Furthermore, viroid titers measured at both 15 and 37 dpi were significantly lower in UC/Ma plants compared to UC/UC, UC, and Ma, suggesting a suppressive effect of UC/Ma grafting on viroid accumulation. These preliminary findings highlight the potential of integrating phenotyping and transcriptomic approaches to unravel tomato defense mechanisms against viroid infections and to evaluate the protective role of grafting.

This research was supported by European Union-NextGeneration EU, Missione 4, Componente 2, investimento 1.1 'progetti di ricerca di rilevante interesse nazionale (PRIN), project PRIN-2022 - DiVInGraft (2022BZW9PF; CUP B53D23017480006)

Poster 5

Effects of hop latent viroid infection on agronomic and biochemical traits of hops

T. G. Zambiassi[1,2], E. A. P. De Paula[1], C. Deschamps[1], R. R. Cipriano[1], K. S. S. Dias[2], A. F. Ramos[2], R. F. Calegario[1], M. Eiras[2]

[1]Departamento de Fitotecnia e Fitossanidade, Universidade Federal do Paraná, Rua dos Funcionários, 1540, 80035-050 Curitiba, Paraná, Brazil; [2]Centro de Pesquisa de Sanidade Vegetal, Instituto Biológico, Av. Conselheiro Rodrigues Alves, 1252, 04016-035 São Paulo, SP, Brazil. *This work was supported by scientific research projects given by CNPq (proc. 401753/2023-9) and FAPESP (proc. 2023/07870-2). TGZ is supported by a technical training fellowship (TT-3) from FAPESP (proc. 2024/07253-6).
E-mail: tzambiassi@ufpr.br; marcelo.eiras@sp.gov.br.

Hop latent viroid (HLVd, *Cocadviroid latenshumuli*, genus *Cocadviroid*, family *Pospiviroidae*) is a globally distributed pathogen of hop (*Humulus lupulus*, Cannabaceae) that normally does not induce symptoms. However, it modifies the chemical composition of infected plants, leading to alterations in compounds essential to the brewing and pharmaceutical industries. HLVd-infected hops may exhibit physiological changes associated with the secretion of metabolites such as alpha- and beta-acids and essential oils, and reductions in yield and bitter acid levels, which may vary depending on the cultivar, an effect clearly dependent on the genotype. HLVd-induced pathogenesis may also disrupt redox processes involved in terpene accumulation. These alterations in the chemical composition of hop cones are not only genotype-dependent but also cultivar-specific, as previously observed in the linalool content of cones from HLVd-infected plants. The present study aimed to evaluate the impact of HLVd infection on agronomic and biochemical traits in hop plants cultivated in South of Brazil.

The experiment was carried out under greenhouse conditions with 22 hop plants of the 'Alpha-Aroma' cultivar. Based on RT-PCR analysis with HLVd-specific primers, which amplified 256 bp DNA fragments, and confirmed by sequencing, 11 plants were identified as infected with HLVd, while the remaining 11 plants were confirmed to be HLVd-free. Agronomic traits evaluated included the number and length of primary branches, as well as fresh and dry biomass of leaves and branches. Biochemical parameters were assessed by quantifying 'chlorophyll a', 'chlorophyll b', total chlorophyll, total sugars, reducing sugars, proteins, and free amino acids in the leaves by spectrophotometry. Our preliminary results revealed an increase in 'chlorophyll a' content in HLVd-infected plants compared to non-infected ones (Student's *t*-test, $p = 0.046$), along with marginal trends toward higher values for total chlorophyll ($p = 0.094$) and branch length ($p = 0.053$) in the infected plants. These preliminary findings suggest that HLVd infection may induce specific metabolic alterations in hop plants, highlighting the need for further research to better understand both its impact on hop production and on the desired properties for the brewing industry.

Poster 6

Hop stunt viroid infecting fig (*Ficus carica* L.) trees in Türkiye

H. K. Ceren[1], N. Buzkan[2]

[1]Science Institute, Kahramanmaraş Sütçü Imam University, Kahramanmaraş, TÜRKİYE; [2]Plant Protection Department, Agriculture Faculty, Kahramanmaraş Sütçü Imam University, Kahramanmaraş, TÜRKİYE.
E-mail: nbuzkan@ksu.edu.tr; buzkan@hotmail.com

Türkiye is one of the most important genetic origins of fig (*Ficus carica* L.) in the world, and it extended to the Mediterranean countries. Fig germplasm in Türkiye is located mainly at the Big Meander Valley and Small Meander Valley in the Aegean Region but also widely seen in the Southeast Anatolia, the Marmara, and the Mediterranean regions. Edible fig trees are known to be natural hosts of several viruses, and their sanitary status has been extensively studied with particular reference to fig mosaic disease and its epidemiology. The incidence of viroids in fig is largely unknown due to a very small number of viroid-specific surveys. There are three viroids including hop stunt viroid (HSVd), citrus exocortis viroid (CEVd) and apple dimple fruit viroid (ADFVd) identified in fig, all belonging to the family *Pospiviroidae*. ADFVd has been the only one identified in fig trees in Turkiye so far. This study was carried out to detect HSVd in fig trees exhibiting fruit and foliar symptoms in varietal collection plot at Sutcu Imam University in Kahramanmaras. Leaf samples from a total of 77 trees were collected to run total nucleic acid (TNA) isolation with modified silica-capture protocol followed by reverse transcription with random hexamer and cDNA synthesis using Maloney Murine Leukemia Virus (M-MLV) reverse transcriptase. PCR was performed in a 50 µl volume reaction with the presence of the oligonucleotides VP-19 and VP-20. Fourteen samples were found with HSVd infection. PCR amplicons were directly sent to Sanger sequencing and analysed with MEGAX software. The Balstn analysis of 271-304 nt sequences revelaed that the highest nucleotide homology of the HSVd-fig variants in fig germplasm was about 94% with the HSVd-pistachio variant from Türkiye. The sequences of the HSVd-fig variants were quite variable among themselves.

The research was granted by the Scientific Research Projects Coordination Unit at Kahramanmaras Sütçü Imam University (#2025/2-15 YLS).

Poster 7

Characterization of citrus viroid VII population diversity across citrus hosts using amplicon sequencing

G.A. Chambers[1,2], A.D.W. Geering[2], D.R. Bogema[1], P. Holford[3], G. Vidalakis[4], N.J. Donovan[1]

[1]Department of Primary Industries and Regional Development, Elizabeth Macarthur Agricultural Institute, New South Wales 2567, Australia; [2]Queensland Alliance for Agriculture and Food, The University of Queensland, Dutton Park, Queensland 4001, Australia; [3]Western Sydney University, Penrith 2751, New South Wales, Australia; [4]University of California, Riverside, CA 92521, USA.
E-mail: grant.chambers@dpi.nsw.gov.au

Viroids are known to form genetically diverse populations within infected plants and this study investigated the population dynamics of citrus viroid VII (CVd-VII, *Apscaviroid etacitri*), a recently described member of the genus *Apscaviroid*, via the application of high-throughput amplicon sequencing. Across three experiments—within-plant (lemon), between-plant (citron), and between-host (five citrus varieties)—62 unique CVd-VII sequence variants were detected. Each sample harbored 2–13 variants, typically with 1–4 major variants (>10% abundance). Single-nucleotide polymorphisms (SNPs), mostly substitutions, accounted for ~10% of the genome variation and were concentrated in the terminal and pathogenic domains. Structural modeling revealed that SNPs influenced the viroid's predicted secondary structure, particularly in the terminal right domain, producing either rod-shaped or bifurcated motifs. In addition, specific variants dominated new flush tissue, host-dependent differences were evident, and inoculated citron plants exhibited unique variant profiles, some distinct from the inoculum source. Interspecies analysis revealed further divergence, suggesting selection pressures, polymerase error rates, or founder effects shape CVd-VII variability. These findings support the quasispecies model for CVd-VII and highlight implications for molecular diagnostics, as some SNPs occurred in primer binding sites. In addition, the study provides foundational knowledge of CVd-VII genetic diversity and paves the way for further investigations on variant-specific pathogenicity and host interactions.

Poster 8

First detection and molecular characterization of apple hammerhead viroid (AHVd) in Tunisian apple orchards

I. Hamdi[1], R. Soltani[2], A. Najar[1]

[1]INRAT (Institut National de la Recherche Agronomique de Tunisie), Rue Hédi Karray, 1004; Ariana - TUNISIE. Laboratoire Protection des Végétaux; [2]CRRA Sidi Bouzid (Centre Régional de Recherche Agricole de Sidi Bouzid), BP: 357 Route Gafsa Km 6, Sidi Bouzid 9100, Tunisie.
E-mail: imenhamdi@yahoo.fr; imen.hamdi@iresa.agrinet.tn

Apple hammerhead viroid (AHVd) is an emerging pathogen of the *Avsunviroidae* family (*Pelamoviroid* genus) that poses a growing threat to global apple production. First identified in China by Zhang et al. (2014) through high-throughput sequencing, AHVd infection is associated with severe symptoms and potential tree decline. Its presence has since been reported in the United States, Japan, Italy, Spain, New Zealand, Montenegro and Germany (Szostek et al., 2018; Zindovic et al., 2024; Zikeli et al., 2025). To assess its potential spread to North Africa, surveys were conducted in Tunisian apple orchards, particularly in the Kasserine region, where significant economic losses have been observed. RT-PCR assays confirmed AHVd infection in 46% of the samples (23/50), indicating a high incidence. These findings suggest that the actual distribution of AHVd in Tunisia is likely underestimated, due to limited surveillance and the absence of systematic viroid screening. Three novel variants were identified, with genome sizes ranging from 433 to 435 nucleotides. Compared to the Chinese reference isolate (KR605506), these variants showed 86–93% sequence identity and strong similarity with the Canadian strain SD17-142. Phylogenetic analysis revealed that the Tunisian isolates clustered into two distinct clades: two variants (AHVd-1 and AHVd-2) grouped with North American strains, while the third (AHVd-3) clustered with Asian and European isolates. This structure suggests multiple introductions or divergent evolutionary pathways. Population genetics analysis using DnaSP further confirmed this diversity. Each genome represented a unique haplotype (Hd = 1.000), with 99 polymorphic sites and a nucleotide diversity (π = 0, 16035), supporting the existence of genetically distinct AHVd lineages in Tunisia. This study provides the first molecular evidence of AHVd in Tunisia and highlights the urgent need for reinforced surveillance and phytosanitary controls to prevent its further spread and reduce its potential economic impact on apple orchards.

Poster 9

Unmasking the hidden virome of carob (*Ceratonia siliqua*)

A. Foteinou[1], A. C. Bibi[2], G. Papagiannakis[2], P. Topalis[2], D. Michelaki[3], P. Ioannidis[4], C. Bazakos[5], N. Kryovrysanaki[2], G. Emvalomatis[3], K. Kalantidis[1,2], K. Katsarou[1.2]

[1]Department of Biology, University of Crete, Voutes University Campus, Heraklion, Crete, Greece; [2]Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, Heraklion, Crete, Greece; [3]Department of Economics, University of Crete, Rethymno, Greece; [4]Institute of Computer Science, Foundation for Research and Technology – Hellas, Heraklion, Crete, Greece; [5]Institute of Plant Breeding and Genetic Resources, Hellenic Agricultural Organization (ELGO) DIMITRA, Thessaloniki-Thermi, Greece
E-mail: katsarou@imbb.forth.gr

Carob (*Ceratonia siligua*) is an important yet underestimated tree of the Mediterranean basin. It has been used by ancient Greeks and Arabs as a common sweetener and helped people survive during periods of great famine. Carob trees have various interesting characteristics, such as drought and temperature tolerance, preventing soil erosion and with the potential to contribute to the development of rural areas. From a nutritional point of view, carob products are rich in sucrose and protein, but poor in fat, therefore are often considered a 'super food'.
Greece is considered an important carob producer, yet due to various reasons, including the development of the tourism industry and the decrease in the number of people choosing farming as a career path, the cultivation is decreasing. In the past few years, there has been an ongoing effort to re-vitalise carob cultivation, mostly by few carob cultivators and cultural associations.
Until today, there is little information about the genome of carob as well as the pathogens affecting carob trees. A few fungi have been identified, all described in Italy in 1998, 2011, and 2013, respectively. However, to our knowledge, there isn't any study available about viral and viroidal infections in carob trees.
In this work, we are presenting the full sequencing of the genome of a carob tree using PacBio technology. In addition, we have collected 134 samples from 34 producers in all four departments of the island of Crete. By HTS, we have identified an important number of viruses and viroids present in these trees. This is the first time that such a global analysis has been conducted for carob. This information is valuable for the promotion of the carob cultivation not only in the island of Crete but worldwide.

Poster 10

Varietal differences in symptom development of CBCVd infection in German hops

C. Krönauer, I. Raith, S. Euringer

Bavarian State Research Center for Agriculture, Institute for Crop Science and Plant Breeding, Hop Research Center, Hüll 5 1/3, 85283 Wolnzach, Germany.
E-mail: christina.kroenauer@lfl.bayern.de

The Hallertau region in the south of Germany is known for its extensive hop-growing area. Hops (*Humulus lupulus*) are cultivated for their cones which are used as a flavoring agent in beer brewing. Hop plants are dioecous and can grow up to seven meters in height on special trellises in agricultural cultivation. In 2019 qPCR analysis identified Citrus bark cracking viroid (CBCVd, recent taxonomic nomenclature: *Codadviroid rimocitri*) as the causal agent of a disease that caused conspicuously malformed plant habitus. CBCVd belongs to the family of *Pospiviroidae* and is a single stranded, self-replicating circular RNA that adopts a rod-like conformation. In hops, it is currently found in Slovenia, Germany and Brazil. Symptoms of CBCVd infection include severe stunting, reduced internode length, chlorosis and abnormally formed cones. Ultimately, CBCVd causes a major reduction in yield and leads to plant death. Combating CBCVd infection in hops is difficult. Hop plants are perennial and cultivation involves numerous techniques that require plant contact and sap transfer, such as crowning and training which facilitate viroid transmission from plant to plant. Furthermore, CBCVd infections often remain latent and visible symptoms appearing late in the season, around mid-July. Symptom development is dependent on cultivar and weather conditions. A variety garden was planted in 2023 in southern Germany, to monitor disease development and identify putative tolerant cultivars. In total, 24 varieties – including those from the breeding program of the Hüll Research Center and cultivars used in hop production – were planted in the field and exposed to CBCVd-infected neighboring plants. Hop plants of each cultivar were distributed in triplicate within the trial field to compensate for differences in location and soil. Infection status and symptom development were recorded in 2025. We observed that the majority of chosen plants were susceptible, while some showed moderate or good tolerance towards CBCVd. The study provides insight into the tolerance levels of German hop varieties to CBCVd and thus offers important information for future decisions regarding the choice of breeding material.

Poster 11

Uncovering viral and viroid diversity in crop – weed interactions in vegetables and citrus agroecosystems

N. Kryovrysanaki[1], G. Papadogiannakis[1], P. Topalis[1], K. Manousoudaki[1], K. Katsarou[1], C. Andronis[1], K. Kalantidis[1, 2]

[1]Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology, Greece, GR-71110 Heraklion/Crete, Greece; [2]Department of Biology, University of Crete, Vassilika Vouton, GR-71409 Heraklion/Crete, Greece.
E-mail: nkrio@imbb.forth.gr

Virus and viroid diseases are of paramount importance in agriculture, affecting both perennial and annual crops such as citrus fruits and vegetables, often leading to significant phytosanitary and economic losses. While viral impact on crop health is well documented, their diversity in non-crop plants and their roles in causing disease is less studied. In the field, cultivated plants coexist with wild or spontaneous flora, creating a dynamic environment where viral exchange can occur. This interaction facilitates the transmission and revival of known and novel viral strains, with wild species potentially acting as reservoirs for future epidemics. Additionally, climate change is altering the distribution patterns of both virus hosts and vectors, intensifying epidemiological pressures. In this study, we conducted a high-throughput sequencing (HTS)-based survey to detect viruses and viroids in vegetable and citrus crops, as well as in various weed species lying adjacent to these crops, across multiple regions of Crete, Greece. Our findings revealed a broad range of plant viruses and viroids, highlighting substantial viral diversity within weed populations. The data will provide evidence regarding the possibility of cultivar-related preferences in pathogen composition, as well as the patterns of mixed infections. The presence of viral and viroid species in economically important crops and associated weeds, along with their effects on host fitness, will be examined to provide insights into the complex interactions between cultivated plants and surrounding flora.

Poster 12

Investigating seed and pollen transmission of hop latent viroid in hops

M. Luigi[1], A. Taglienti[1], T. Ganino[2], M. Rodolfi[2], T. Lino[2], K. Carbone[3], F. Faggioli[1], L. Ferretti[1]

[1]CREA Centro di Ricerca Difesa e Certificazione (CREA-DC), Via C. G. Bertero 22, 00156 Roma, Italy; [2]Università di Parma, Dipartimento di Scienze degli Alimenti e del Farmaco, Parco Area delle Scienze 11/A, 43124 Parma, Italy; [3]CREA Centro di Ricerca Olivicoltura, Frutticoltura e Agrumicoltura (CREA-OFA), Via di Fioranello 52, 00134 Roma, Italy.
E-mail: marta.luigi@crea.gov.it

Rates of vertical transmission of viroids have always been a controversial topic over the years. Pollen transmission has been described for viroids belonging both to the *Avsuvviroidae* and *Pospiviroidae* family, whereas seeds transmission seems to be strictly linked to the host/viroid combination (Hadidi *et al*., 2022). Hop latent viroid (HLVd - *Cocadviroid latenshumuli*; genus *Cocadviroid* family *Pospiviroidae*) is a pathogen that mainly infects hop (*Humulus lupulus*) but also hemp plants (*Cannabis sativa*) often without causing visible symptoms — hence the term *latent*. However, it can impact yield and quality, especially in commercial hop production with potential transmission routes that include vegetative propagation, pollen, and possibly seeds. In hemp, female parental plants pollinated with pollen obtained from HLVd-infected male parents were found infected, along with 58% of obtained seedlings, confirming a potential role of pollen in vertical transmission (Atallah et al., 2023). Similar tests on hemp were conducted by Punja et al. (2025), showing a transmission rate of 100% either by pollen or seeds. Regarding hops, the study by Matoušek et al. (2000) reports a very low or negligible seed transmission rate, to the extent that seed propagation is recommended as a method for 'cleaning up' hop germplasm, given the widespread presence of HLVd in hop orchards. In Italy, the presence of HLVd was ascertained in almost all the cultivated hop plants tested (Gargani *et al*., 2018) confirming the widespread presence of the viroid. To further investigate the potential seed and pollen transmission of HLVd in hops, female parental plants and pollen used in a controlled crossbreeding program, were molecularly investigated. Specifically, pollen from cv. Chinook was collected, separated from anthers and total RNA (TRNA) was extracted from 200 mg fresh weight. Leaves from female recipient plants cv. Chinook and Comet were collected and analyzed as well. The TRNA from both pollen and leaf tissue was analyzed by a HLVd-specific RT-PCR-based test, which assessed the presence of HLVd in the pollen and in both the female parent plants. Then, 420 F1 hybrid seedlings generated from the female parental plant were sampled, pooled in groups of ten and analyzed. None of the pools tested positive for the presence of HLVd. According to these preliminary results, the transmission of HLVd in hop seems to involve at least only pollen and not seeds. Specific transmission trials involving healthy female plants and HLVd-infected male plants acting as pollen donors, performed under controlled growing conditions, are ongoing in order to better evaluate the role played by pollen in the transmission of HLVd and in its widespread presence in most of the commercial hop orchards in Italy.

This study was carried out in the frame of the project “Luppolo, Orzo, Birra: biodiversità ITaliana da valorizzare - LOB.IT” financed by the Italian Ministry of Agriculture (Masaf, D.M. n. 667550, 30.12.2022).

Atallah OO, Yassin SM, Verchot J. New Insights into Hop Latent Viroid Detection, Infectivity, Host Range, and Transmission. Viruses. 2023 Dec 23;16(1):30. doi: 10.3390/v16010030. PMID: 38257731; PMCID: PMC10819085.
Gargani, E., Ferretti, L., Faggioli, F., Haegi, A., Luigi, M., Landi, S., Simoni, S., Benvenuti, C., Guidi, S., Simoncini, S., D'Errico, G., Amoriello, T., Ciccoritti, R., Roversi, P.F. and Carbone, K. (2017) 'A survey on pests and diseases of Italian Hop crops', Italus Hortus, 24(2), pp. 1-17. doi: 10.26353/j.itahort/2017.2.117.
Hadidi, A., Sun, L., Randles, J.W. 2022. Modes of Viroid Transmission. Cells 2022, 11, 719. https://doi.org/10.3390/cells1104071
Punja, Z.K.; Scott, C.; Tso, H.H.; Munz, J.; Buirs, L. Transmission, Spread, Longevity and Management of Hop Latent Viroid, a Widespread and Destructive Pathogen Affecting Cannabis (Cannabis sativa L.) Plants in North America. Plants 2025, 14, 830. https://doi.org/10.3390/plants14050830.

Poster 13

Rapid and field-deployable detection of viruses and viroids in tomato using LAMP directly from crude plant extracts

K. Manousoudaki[1], A. James[1], K. Katsarou[1], K. Kalantidis[1,2], N. Kryovrysanaki[1]

[1]Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology, Heraklion, Crete, Greece; [2]Department of Biology, University of Crete, Heraklion, Crete, Greece.
E-mail: claire.manous@outlook.com; kriton@imbb.forth.gr

Tomato (*Solanum lycopersicum*) is a globally important crop, frequently affected by a wide range of viral and viroid pathogens that threaten productivity and economy. Early and accurate detection of viruses and viroids is essential for effective disease management and epidemiological monitoring. Traditional molecular diagnostic methods, such as PCR and qPCR, although sensitive and specific, require specialized equipment, trained personnel and nucleic acid purification steps that limit their use in field settings. In recent years, Loop-mediated Isothermal Amplification (LAMP) has emerged as a powerful alternative for the rapid, sensitive, and cost-effective detection of plant pathogens. In this study, we grew an application of LAMP assays for the detection of economically important tomato-infecting viruses and viroids directly from crude plant extracts, bypassing the need for RNA/DNA extraction and cover all the ASSURED criteria that World Health Organization has developed for a point-of-care diagnostic test. The approach enables amplification at a constant temperature using a simple wireless, heating device and the results can be visualized through colorimetric-based readouts into a real-time connectivity through a wireless system. We optimized crude extraction protocols for leaf tissue and validated LAMP assays targeting representative viral and viroid genomes, demonstrating high sensitivity and specificity comparable to conventional methods. Our findings support the potential of LAMP as a field-deployable diagnostic tool for the early detection of tomato pathogens, contributing to rapid decision-making in crop protection and plant health surveillance frameworks.

Poster 14

Eradication of hop latent viroid (HLVd) in a medical cannabis seed bank using dot-blot hybridization and phytosanitary measures

P. Serra, G. Corrado, E. Llosa, J.A. Sanchez-Navarro, V. Pallás

Institute of Molecular and Cellular Biology of Plants, Polytechnic University of Valencia -Spanish National Research Council (CSIC), Valencia, Spain.
E-mail: pederal@upvnet.upv.es; vpallas@ibmcp.upv.es.

In the past decade, the medical and recreational cannabis industry has experienced rapid growth as many countries have relaxed restrictions on its cultivation to obtain cannabinoids, the most important of which are cannabidiol (CBD) and Δ9-tetrahydrocannabinol (THC). The appearance of large plantations has been accompanied by the incidence of various pathologies, the most serious of which is caused by hop latent viroid (HLVd). This subviral agent often produces no obvious symptoms but can lead to significant reductions in the production of flowers, trichrome development and concentration of cannabinoids.
The lack of experience in the use of phytosanitary measures in the sector has resulted in widespread viroid infection within seed and germplasm banks that maintain elite clones for large-scale propagation. RT-PCR studies indicate that HLVd in cannabis has a very high transmission both horizontally (via pruning tools and via irrigation water), and vertically with transmission rates reaching 60% (pollen) and 80% from infected mother plants.
This work describes the eradication of HLVd in a medical cannabis seed bank using dot-blot hybridization and the application of sanitary measures in the handling of the plants. For this purpose, we (i) have tested and fine-tuned molecular dot-blot hybridization and tissue printing for reliable detection of HLVd, (ii) have determined the incubation times required to detect HLVd in roots and aerial part by both RT-PCR and molecular dot blot hybridization establishing effective quarantine protocols for new plant material, (iii) implemented disinfection procedures for pruning tools and isolation measures to prevent cross-contamination via irrigation and (iv) conducted large-scale screenings to eliminate infected material and, in some cases, propagate healthy clones from plants with early root detection.
With these measures we have cleaned up the variety bank that started with more than 60% of plants infected by HLVd, demonstrating that dot-blot hybridization from citrate buffer extractions is a valid, fast, cost-effective, and scalable method for routine HLVd diagnosis, essential for the management and recovery of high-value cannabis germplasm collections.

Poster 15

Detection of pospiviroids in solanaceous seed species collected from Africa

A. Skelton[1], A. Fowkes[1], L. Frew[1], J. Murphy[1], M. Mynett[1], O. Wagstaff[2], T.R. Pearce[2], C. Cockel[2], D.O. Nyamongo[3], J.M. Wasswa[4], A. Fox[1]

[1]Fera Science Ltd., Sand Hutton, York, YO41 1LZ; [2]The Royal Botanic Gardens, Kew, Millenium Seed Bank, Wakehurst Place, Ardingly, West Sussex, RH17 6TN; [3]Kenya Genetic Resources Research Institute, Nairobi, Kenya; [4]Plant Genetic Resources Centre, Entebbe, Uganda
E-mail: anna.skelton@fera.co.uk

In 2016 seed of wild solanaceous species related to *Solanum melongena* (aubergine) were collected from several African countries as part of the Adapting Agriculture to Climate Change (Crop Wild Relatives) project, managed by the Global Crop Diversity Trust and the Millennium Seed Bank, Royal Botanic Gardens, Kew (RBG Kew). The solanaceous species collected grow wild in Africa and are used for food and/or medicinal purposes.
In 2018, to allow export to a research centre in Taiwan, samples of the seed were sent to Fera Science Ltd from RBG Kew, for viroid testing. Seed of *S. anguivi*, *S. coagulans* and *S. dasyphyllum,* from Ghana, Kenya and Uganda, tested positive by real-time RT-PCR for potato spindle tuber viroid (PSTVd). This was the first report of a pospiviroid being detected in these hosts, confirming the potential for pospiviroids to be distributed through non-commercial seed (Skelton, 2019). There have been very few reports of PSTVd in Africa; with previous limited findings in north and west Africa (EPPO global database). This testing indicated that PSTVd may have been more widely distributed than suggested by reports.
In 2019, a further batch of solanaceous seed species from Africa were sent from RBG Kew to Fera, again to facilitate export for crop improvement breeding. These were tested for a range of pospiviroids by real-time RT-PCR, including PSTVd, Columnea latent viroid and tomato apical stunt viroid. Several pospiviroids were detected in different solanaceous hosts, including TASVd in a new host (*S. macrocarpon*) and a new country finding for CLVd (Sudan). A couple of the seed samples were also tested further by high throughput sequencing. These results will be discussed.

References

EPPO/OEPP. Potato spindle tuber viroid, EPPO Global Database. 2025. Available online: https://gd.eppo.int/taxon/PSTVD0/distribution (accessed on 19 June 2025).
Skelton, A., et al. "Potato spindle tuber viroid detected in seed of uncultivated Solanum anguivi, S. coagulans and S. dasyphyllum collected from Ghana, Kenya and Uganda." New Dis. Rep 39.23 (2019): 2044-0588.

Acknowledgements

This testing was funded through the Defra-Fera Long Term Service Agreement. The Adapting Agriculture to Climate Change (Crop Wild Relatives) project is funded by the Government of Norway.

Poster 16

Chasing AHVd across Western Balkans – Croatian and Montenegrin examples

J. Zindović[1], V. Miljanić[2], N. Štajner[2], J. Jakše[2], J. Matijević[3], D. Škorić[3]

[1]Biotechnical Faculty, University of Montenegro, Mihajla Lalića 15, 81000 Podgorica, Montenegro; [2]Agronomy Department, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, 1000 Ljubljana, Slovenia; [3]Department of Biology, Faculty of Science, University of Zagreb, Marulićev trg 9a, 10000 Zagreb. E-mail: jelenazindovic@yahoo.com; dijana.skoric@biol.pmf.unizg.hr

Apple hammerhead viroid (AHVd, *Pelamoviroid malleusmali*, *Avsunviroidae*) is increasingly reported from apple orchards worldwide, though its economic impact remains unclear. To assess its occurrence in the Western Balkans, targeted surveys were carried out in Montenegro (2020–2022) and Croatia (2023), revealing contrasting sampling contexts and shared challenges in molecular detection.

In Montenegro, 29 symptomatic apple leaf samples were collected from 16 locations across 7 municipalities, covering a range of tree ages (15–40 years) and cultivars, from indigenous landraces to internationally cultivated varieties. Nucleic acids were extracted from leaves, primarily by RNeasy Plant mini kit (Qiagen) and a minority by CTAB based protocol. AHVd detection was performed by RT-PCR using primer pair AHVd13F_PG/12R_PG (Messmer *et al.* 2017), and yielded five positive samples from four localities across four different municipalities. Full or partial genomes were obtained from three apple trees by Sanger sequencing and *in silico* assembly (GenBank acc. nos. PP133245–47). These isolates exhibited typical hammerhead motifs and had a nucleotide identity of 92.3–98.8% with reference isolates from Europe and North America. A snapshot of the AHVd status in apples was conducted in Croatia in 2023. The analyses were performed on two asymptomatic fruit samples from a county where apples are not grown commercially while 15 archived CTAB leaf phloem extracts from apples with apple proliferation symptoms and proven phytoplasma infection were included from districts with intensive cultivation Additionally, two symptomatic fruits bought for consumption at a grocery store were tested. RT-PCR using AHVd13F_PG/12R_PG yielded amplicons in four archival leaf samples, one out of two apples marketed for consumption, and both asymptomatic fruit samples. However, the use of other primers (e.g., AHVd RF-1379/1380 from Serra *et al.* 2018) intended to obtain a complete viroid sequence was not successful in most cases. Partial sequences of the viroid were obtained (OQ921334-45, OR043662-65, PV111791). Isolates from Croatia and Montenegro were classified within *Pelamoviroid malleusmali* species and showed conserved hammerhead structures. Comparative analysis using over 200 AHVd sequences in NCBI confirmed their identities. Newly available alignment features in NCBI revealed sequence variation near primer sites—further highlighting the need for improved diagnostics. Notably, a decade-old CTAB leaf extracts and fresh fruit tissue both proved to be useful for detection. In both countries, difficulties in obtaining full-length AHVd sequences by Sanger sequencing underscored a common limitation of standard RT-PCR-based workflows particularly under conditions of low viroid titer and/or sequence divergence at primer binding sites. Besides a haphazard availability of unbiased detection by high-throughput sequencing, these technical obstacles, shared despite different sample types and extraction protocols, suggest a need for refined cost-effective detection strategies, the importance of which must be seen in the light of long-term potential of viroids to affect the health status of the apple trees along with viruses and phytoplasmas.

References: Messmer *et al.* (2017) Can. J. Plant Pathol. 39:342; Serra *et al.* (2018) Virus. Res. 249: 8-15.

Poster 17

Virome analysis of Slovenian grapevines reveals high prevalence of GYSVd-1 and HSVd

V. Miljanić, J. Jakše, N. Štajner

Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, 1000 Ljubljana, Slovenia
E-mail: natasa.stajner@bf.uni-lj.si

Viroids are small, circular, single-stranded, non-coding RNAs that replicate in host plant cells without encoding proteins. In grapevine (*Vitis* spp.), six viroids and one viroid-like RNA have been reported: grapevine yellow speckle viroid 1 (GYSVd-1), GYSVd-2, grapevine latent viroid (GLVd), Australian grapevine viroid (AGVd), hop stunt viroid (HSVd), citrus exocortis viroid (CEVd), and grapevine hammerhead viroid-like RNA (GHVd). GYSVd-1 and HSVd, members of the family *Pospiviroidae*, are globally distributed and the only two viroids detected in grapevines in Slovenia.
HSVd, originally identified in hops, is latent in grapevines but transmissible to other hosts, including hops, where it can cause disease. It has a rod-like genome of 296–302 nt containing a central conserved region (CCR) and a terminal conserved hairpin (TCH). GYSVd-1, associated with yellow speckle disease, also exhibits a rod-like structure with a CCR and terminal conserved region (TCR) and can induce vein banding when co-infected with grapevine fanleaf virus (GFLV).
To investigate viroid prevalence, small RNA sequencing (sRNA-seq) was performed on grapevine samples from the Ampelographic collection Kromberk, Slovenia, followed by validation using multiplex RT-PCR (mRT-PCR). Among a broad virome that included nine viruses, both HSVd and GYSVd-1 were consistently detected. HSVd was found in all 79 samples and confirmed by RT-PCR using primers HSV-78P/HSV-83M. Sequencing of 40 isolates revealed low diversity, with 38 identical sequences and two divergent isolates clustering separately in phylogenetic analysis. In contrast, GYSVd-1 showed higher sequence variability, including InDel mutations, and was validated in 71 samples (89.87%) using whole-genome primers. Co-infection of GYSVd-1 with GFLV was found in several samples (two of ‘Zeleni Sauvignon’ variety and five of ‘Pokalca’ variety), though no visible symptoms were observed. Phylogenetic analysis of 35 Slovenian GYSVd-1 isolates generated in this study and 28 sequences from database showed that our isolates clustered in different phylogroups, independently of variety or geographic distribution.
In a complementary study, thirteen samples from six grapevine varieties ('Malvazija', 'Rebula', 'Pokalca', 'Cipro', 'Volovnik' and 'Poljšakica') not included in clonal selection were analyzed. sRNA-seq of four libraries revealed a similar virome, with HSVd detected in all and GYSVd-1 absent in one. Multiplex reverse transcription-polymerase chain reaction (mRT-PCR) was developed for validation of sRNA-seq predicted infections, including various combinations of viruses or viroids and satellite RNA. These results confirm the widespread, often asymptomatic presence of HSVd and GYSVd-1 in Slovenian grapevines and underscore the importance of sensitive molecular tools, such as sRNA-seq and mRT-PCR, for viroid diagnostics and certification programs.

References:

MILJANIĆ, V., JAKŠE, J., ŠTAJNER, N. et al. Virome status of preclonal candidates of grapevine varieties (Vitis vinifera L.) from the Slovenian wine-growing region Primorska as determined by high-throughput sequencing. *Frontiers in microbiology*. 2022

MILJANIĆ, V, JAKŠE, J., ŠTAJNER, N. et al. Small RNA sequencing and multiplex RT-PCR for diagnostics of grapevine viruses and virus-like organisms. *Viruses*. 2022

Poster 18

Armillaria ambiviruses: a potential tool for biocontrol of forest pathogens

B. Opelka[1], T. Tonka[2]

University of South Bohemia in Ceske Budejovice, Faculty of Agriculture and Technology, Studentska 1668, 370 05 Ceske Budejovice, Czech Republic.
Deans Secretariat E-mail: strobova@fzt.jcu.cz, Autors E-mail: pichab00@fzt.jcu.cz

Armillaria ostoyae is a major fungal pathogen responsible for *root rot disease* in coniferous forests, leading to significant ecological and economic losses. Recent studies suggest that mycoviruses associated with *Armillaria* species may influence fungal virulence and could serve as potential biocontrol agents. This study aimed to identify and characterize mycoviruses in *A. ostoyae* isolates collected across the Czech Republic. Fungal samples were cultivated on malt extract agar, and total RNA was extracted for next-generation sequencing using the *Illumina NovaSeq 6000* platform. Bioinformatics analysis revealed novel viral sequences, including ssRNA viruses related to *Ambiviridae* and *Benyviridae*, and dsRNA viruses from the *Partitiviridae* family. Ambi-like virus genome (~4596 nt) showed 78% nucleotide and 99% protein similarity to Armillaria ambi-like virus *3*. Presence of virus was confirmed in multiple samples using PCR and Sanger sequencing. To enhance detection sensitivity, quantitative real-time PCR (qPCR) was employed using SYBR™ Green system and specific-designed primers. qPCR detected viral RNA in additional samples that tested negative by conventional PCR, demonstrating the method's superior sensitivity. These findings expand the known diversity of mycoviruses in *A. ostoyae* and support the potential application of hypovirulence-associated viruses in forest disease management. Further research will focus on functional characterization and estimate of their biocontrol potential.

Poster 19

Ectopic expression and subcellular localization of the putative RNA-dependent RNA polymerase of Tulasnella ambivirus 4 in *Saccharomyces cerevisiae*

N. Serale[1], S. Daghino[2], M. Forgia[1], F. Di Serio[2], B. Navarro[1], L. Rubino[1]

[1]Institute for Sustainable Plant Protection, National Research Council of Italy, Via Amendola 165/a, 70126, Bari, Italy; [2]Institute for Sustainable Plant Protection, National Research Council of Italy, Strada delle Cacce, 73, 10135 Turin, Italy.
E-mail: luisa.rubino@cnr.it

Ambiviruses are infectious agents possessing a circular single-stranded RNA genome folding in a compact secondary structure and with paired self-cleaving ribozymes, which replicates through a rolling circle mechanism. Besides presenting typical features of viroid-like RNAs, ambiviruses share some characteristics of RNA viruses. They harbour two open reading frames (ORFs; ORFA and ORFB) which code for one protein in each polarity strand. ORFB encode a protein with unknown function. ORFA potentially code for an RNA-dependent RNA polymerase (RdRP), based on structural similarity to viral RdRPs, as shown by structural and phylogenetic analysis. Nevertheless, its role remains to be elucidated. Here, to investigate the function of ORFA gene product, we used Tulasnella ambivirus 4 (TuAmV4) as a model, which infects *Tulasnella* sp., mycorrhizal fungi of orchid roots. The budding yeast *Saccharomyces cerevisiae* was selected as a surrogate model host, since it has been successfully used to express non-structural viral proteins to study their subcellular localization and the signals targeting to specific cell membranes.
TuAmV4 ORFA fused to the Myc epitope was cloned under the control of a constitutive promoter in the yeast expression vector pA (pA-TuAmV4ORFAMyc) or of an inducible promoter in the yeast expression vector pYES2 (pYES2-TuAmV4ORFAMyc). Both constructs were used to transform *S. cerevisiae* YPH499 cells. The optimal culture conditions for the protein expression were determined. TuAmV4 ORFA was correctly expressed either constitutively or transiently, as shown by western blot analysis. The ectopic expression of ORFA did not affect yeast cell growth as a function of time. By differential centrifugation of protein extracts it was shown that ORFA expressed protein sedimented in a membrane-enriched fraction. Furthermore, TuAmV4 ORFA product resulted to be resistant to alkaline, urea or salt extraction, a property of integral membrane proteins. Finally, we observed the localization of the protein in yeast cells by immunofluorescence analysis. Results showed that the TuAmV4 ORFA was not dispersed in the cytosol, but it co-localized with a marker of the endoplasmic reticulum.

This research was funded by European Union - Next Generation EU, Missione 4, Componente 2, project PRIN-2022-PNRR CIRCUFUN (P2022XX55J, CUP B53D23023750001

Poster 20

New infectious agents in the Glomeromycotina: characterization of viroid-like elements in *Rhizophagus* and *Gigaspora* species

P. Mussano[1,2], M. Forgia[2,3], M. Chiumenti[3], S. Daghino[2], A. Crosino[1], B. Navarro[3], N. Serale[3], F. Di Serio[3], M. Turina[2], M. C. Garces-Ruiz[4], S. Declerck[4], L. Lanfranco[1]

[1]Department of Life Sciences and Systems Biology, University of Turin, Italy; [2]Institute for Sustainable Plant Protection, National Research Council of Italy, Turin, Italy; [3]Institute for Sustainable Plant Protection, National Research Council of Italy, Bari, Italy; [4]Earth & Life Institute, Catholic University of Louvain, Louvain-la-Neuve, Walloon Brabant, Belgium.
E-mail: p.mussano@unito.it

Plants live in association with diverse microorganisms ranging from symbiotic to pathogenic. One of the most studied groups of plant symbionts are arbuscular mycorrhizal fungi (AMF), belonging to the Glomeromycotina. These soil fungi are often reported to increase growth and health of plants, playing fundamental roles in nutrition, development and defense. Viroid and viroid-like elements (vdlRNA) have circular RNA-based genomes of different sizes with very distinct characteristics. In this work we looked for the presence of possible vdlRNAs in three AMF strains, two belonging to the *Rhizophagus fasciculatus* species (MUCL58265 and MUCL46100) from the MUCL/GINCO international collection (Louvain-la-Neuve, Belgium) and a *Gigaspora margarita* strain (BEG34). The presence of putative viroid-like elements was assessed from total RNA sequencing data with specific bioinformatic pipelines and then confirmed by contig-specific RTq-PCR in every fungal host. Two viroid-like elements were detected in *R. fasciculatus*, one was conserved in two different isolates while the other one was found exclusively in a single isolate. A third viroid-like RNA was identified in *G. margarita*. These three RNAs are circular RNAs, have a size of about 2 kb and, based on structural prediction analyses, adopt highly branched secondary structures with hammerhead ribozymes in one polarity strand. To ensure that these RNAs molecules do not derive from the transcription of the host genomes, q-PCR was perfomed using DNA as template, yielding negative results.
Further experiments will aim to assess i) the self-cleavage activity of the ribozymes both *in vitro* and *in vivo*, and ii) the accumulation level in the fungal host of both linear and circular forms of these viroid-like RNAs. Our work for the first time associates the presence of viroid-like RNA elements to members of the Glomeromycotina, an early diverging clade of the kingdom Fungi with a long history of co-evolution with plants.

This research was funded by European Union - Next Generation EU, Missione 4, Componente 2, project PRIN-2022-PNRR CIRCUFUN (P2022XX55J, CUP B53D23023750001)

Poster 21

Unveiling hidden ribozyme activities in fungal viroid-like RNAs

N. Serale[1], P. Mussano[2,3], M. Chiumenti[1], M. Forgia[1], S. Daghino[2], M. Turina[2], F. Di Serio[2], B. Navarro[1]

[1]Istituto per la Protezione Sostenibile delle Piante – Consiglio Nazionale delle Ricerche (IPSP-CNR)-SS Bari, Via Amendola 165/A, 70126 Bari, Italy; [2]Istituto per la Protezione Sostenibile delle Piante – Consiglio Nazionale delle Ricerche (IPSP-CNR), Strada delle Cacce, 73, 10135 Torino, Italy; [3]Department of Life Sciences and Systems Biology, University of Turin, Viale Mattioli 25, 10125, Torino, Italy. E-mail: beatriz.navarro@cnr.it

Viroid-like RNAs (vdlRNAs), originally infecting plants and animals, have been recently identified in fungi, bacteria, and in different ecological sources. They consist of infectious circular RNAs that adopt compact conformations and typically harbor paired self-cleaving ribozymes. These ribozymes are involved in the replication via a symmetric rolling-circle mechanism, catalyzing the cleavage of multimeric RNA intermediates of both polarity strands into monomers. To date, hammerhead, hairpin, hepatitis delta, and twister ribozymes have been found in vdlRNAs. Despite the growing number of vdlRNAs identified through *in silico* analyses, only few of them have been molecularly characterized and associated to a host and their biological and ecological roles remain largely unknown. To fill this gap, metatranscriptomic analysis were performed on pools of fungal isolates from collections and circular RNAs carrying ribozymes were identified using structural-based homology-independent bioinformatic tools such as Infernal and vdsearch. The candidate vdlRNAs identified, were associated to specific fungal isolate(s) of the pool, and their circularity and lack of DNA counterparts were assessed by RT-PCR and PCR, respectively. Among the viroid-like RNAs experimentally confirmed, in this study we focused on three novel vdlRNAs each containing a predicted ribozyme on one polarity strand, but none on the opposite strand. In detail, N8423 and N6490 vdlRNAs were identified in two mycorrhizal *Ceratobasidium* sp. strains (AM7_1C and AM7_4C), isolated from the roots of Mediterranean terrestrial orchids in Italy. These circular RNAs with a genome of 1,378 and 1,625 nt, respectively, adopt quasi-rod-like secondary structures and contain a hairpin ribozyme on one strand. N914 vdlRNA was associated with a phytopathogenic *Rhizoctonia* strain (RH-PAO) from potato Sardinian crops. Its 2,472-nt genome forms a highly branched structure with a hammerhead ribozyme on one strand. For all three vdlRNAs, ribozyme self-cleavage activity and cleavage site were validated by *in vitro* transcription and 5'RACE, respectively. Interestingly, in all these vdlRNAs, the polarity strand lacking a known ribozyme also exhibited *in vitro* self-cleaving activity, suggesting the presence of a novel ribozyme not characterized yet. In addition, Northen blot hybridization assays of total RNAs from AM7_1C isolate showing that circular and linear forms of both polarity strands of N8423 vdlRNAs accumulate in the infected fungus, suggest that it replicates through a symmetric rolling circle mechanism, an evidence consistent with the presence of an active self-cleaving ribozyme in each polarity strand. Further experiments have been designed to identify the cleavage sites *in vivo* and *in vitro* and elucidate the structure of these potential new ribozymes.

This research was funded by European Union - Next Generation EU, Missione 4, Componente 2, project PRIN-2022-PNRR CIRCUFUN (number P2022XX55J, CUP B53D23023750001)

Poster 22

Fungal and plant RNA viruses found to exploit self-cleaving ribozymes for translation initiation

M.J. López-Galiano[1], O. Rueda[1], S. Chiba[2], M. Forgia[3], B. Navarro[3], A. Cervera[1], A. Babaian[5], F. Di Serio[4], M. Turina[6], M. de la Peña[1]

[1]Instituto de Biología Molecular y Celular de Plantas, UPV-CSIC, Valencia, Spain; [2]Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya, Japan; [3]Institute for Sustainable Plant Protection, National Research Council of Italy, Bari, Italy; [4]Institute for Sustainable Plant Protection, National Research Council of Italy, Torino, Italy; [5]Department of Molecular Genetics, University of Toronto, Toronto, Canada; [6]Institute for Sustainable Plant Protection, National Research Council of Italy, Brescia, Italy.
E-mail: olruelo@ibmcp.upv.es

Small self-cleaving ribozymes are catalytic RNAs originally discovered in viroid-like agents, which are replicating circular RNAs (circRNAs) postulated as relics of a prebiotic RNA world. In the last decade, however, small ribozymes have also been detected across the tree of life, from bacterial to human genomes, and more recently, in viral agents with circRNA genomes. Here we report the conserved occurrence of diverse small ribozymes within the linear genomes of typical double- and single-stranded RNA virus families from fungi and plants. In most 5'-UTR regions of chrysovirids and fusarivirids, we find conserved hammerhead ribozymes (hhrbzs) showing efficient self-cleaving activity *in vitro* and *in vivo*. Similar hhrbzs, as well as hepatitis delta and twister ribozymes, are also present in megabirna-, hypo-, fusagra- and toti-like viruses. The ribozymes occur not only as isolated motifs within UTRs but also as tandem pairs that encompass short sequences (186-399 nt) with evident resemblance to Zetavirus-like genomes. *In vivo* characterization of the 5' UTR of a fungal chrysovirid containing a hhrbz revealed an unexpected role of RNA cleavage in protein translation. Analogous in vivo experiments done with diverse small ribozyme motifs indicated that self-cleaving activity in the mRNA could induce cap-independent translation through an unknown mechanism. We conclude that small self-cleaving ribozymes, historically linked to the rolling circle replication of viroid-like RNA agents, have been co-opted by diverse linear RNA viruses to perform translational functions.

Poster 23

Designer circRNA$_{GFP}$ reduces GFP-abundance in Arabidopsis protoplasts in a sequence-specific manner, independent of RNAi pathways

M. Hossain[1], C. Pfafenrot[2], S. Nasfi[1], A. Sede[3], J. Imani[1], E. Šečić[1], M. Galli[1], P. Schäfer[1], A. Bindereif[2], M. Heinlein[3], M. Ladera-Carmona[1], K.H. Kogel[1, 3]

[1]Institute of Phytopathology, Centre for BioSystems, Land Use and Nutrition, Justus Liebig University, 35392 Giessen, Germany; [2]Institute of Biochemistry, Justus Liebig University, 35392 Giessen, Germany; [3]Institut de Biologie Moléculaire des Plantes, CNRS, Université de Strasbourg, 67084 Strasbourg, France.
E-mail: manfred.heinlein@ibmp-cnrs.unistra.fr

Circular RNAs (circRNAs) are single-stranded RNA molecules characterized by their covalently closed structure and are emerging as key regulators of cellular processes in mammals, including gene expression, protein function and immune responses. Recent evidence suggests that circRNAs also play significant roles in plants, influencing development, nutrition, biotic stress resistance, and abiotic stress tolerance. However, the potential of circRNAs to modulate target protein abundance in plants remains largely unexplored. In this study, we investigated the potential of designer circRNAs to modulate target protein abundance in plants using Arabidopsis protoplasts as a model system. We show that PEG-mediated transfection with a 50-nt circRNA$_{GFP}$ containing a 30-nt GFP-antisense sequence results in a dose- and sequence-dependent reduction of GFP reporter target protein abundance. Notably, a single-stranded open isoform of circRNA$_{GFP}$ had little effect on protein abundance, indicating the importance of the closed circular structure. Additionally, circRNA$_{GFP}$ also reduced GFP abundance in Arabidopsis mutants defective in RNA interference (RNAi), suggesting that circRNA activity is independent of the RNAi pathway. We also show that circRNA, unlike dsRNA, does not induce pattern-triggered immunity (PTI) in plants. These findings represent crucial first steps in understanding the potential of circRNAs as versatile tools for modulating gene expression and offer exciting prospects for their application in agronomy, particularly for enhancing crop traits through metabolic pathway manipulation.

Reference:
Hossain M, Pfafenrot C, Nasfi S, Sede A, Imani J, Šečić E, Galli M, Schäfer P, Bindereif A, Heinlein M, Ladera-Carmona M, Kogel KH (2025) Designer circRNA$_{GFP}$ reduces GFP-abundance in Arabidopsis protoplasts in a sequence-specific manner, independent of RNAi pathways. *Plant Cell Reports* 44, 128

Poster 24 – DN1

Towards understanding viroid-host interactions through host gene disruption studies

J. Colaço[1], H. Volk[1], J-A. Daròs[2], N. Štajner[1], J. Jakše[1]

[1]Biotechnical Faculty, Department of Agronomy, University of Ljubljana, Jamnikarjeva 101, 1000 Ljubljana, Slovenia [2]Instituto de Biologıa Molecular y Celular de Plantas, Consejo Superior de Investigaciones Cientıficas, Universitat Politecnica de Valencia, 46022 Valencia, Spain.
E-mail: joao.colaco@bf.uni-lj.si

Viroids are the smallest known infectious agents affecting plants. They consist of circular, highly structured RNA molecules, ranging from 246 to 401 nucleotides in length, and notably without any coding potential. The viroid life cycle is therefore completely dependent on the host plant proteins and factors. Some of them are already characterized and they include: a) nuclear import proteins (e.g. VirP1 mediates nuclear import of viroids through importin-based pathway), b) RNA polymerase II machinery (e.g. a splice variant of transcription factor IIIA facilitates viroid replication, with functional organization of RNA Pol-II), c) processing enzymes (e.g. DNA ligase 1 is implicated in viroid circularization) and d) proteins involved in transportation from the nucleus to the cytoplasm and in cell-to-cell movement (potentially via the 5S rRNA export pathway). This doctoral project aims to identify any novel host proteins involved in viroid biogenesis and/or to confirm those already characterized by analyzing gene expression changes during viroid infections across plant species. We aim to collect and analyze publicly available RNA-seq datasets from various plant-viroid experiments to identify common and unique differentially expressed host transcripts and enriched pathways during viroid infection. Up to six candidate host genes will be selected and silenced either in hop (*Humulus lupulus*) or model plant (e.g. *Nicotiana benthamiana*) using virus-induced gene silencing (VIGS), followed by infection with selected viroid species (e.g. CBCVd, *Cocadviroid rimocitri*). After infection, viroid titers and disease symptoms will be monitored over a six-month period. RNA will be extracted at multiple time points post-inoculation to quantify CBCVd levels using RT-qPCR based on TaqMan approach, while disease symptoms and progression will be assessed using a scoring system. This doctoral project will provide new insights into how viroids interact with their host plants and helping to improve our understanding of the molecular processes involved in infection. This knowledge may offer a valuable basis for developing future breeding or preventive strategies to increase viroid resistance in important agricultural crops.

Funded by the European Union within the Horizon Europe MSCA Doctoral Networks, Reference Number HORIZON-MSCA-2023-DN-01, Project ViroiDoc, Grant Agreement Number: 101169421. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

Poster 25 – DN2

Climate change impact on viroid diseases. Application to Citrus bark cracking viroid (CBCVd)- infected hops

O. Lacroix[1], M. Mikulič Petkovšek[2], J. Jakše[2], S. Radišek[1]

[1]Slovenian Institute of Hop Research and Brewing, Cesta Žalskega tabora 2, 3310 Žalec, Slovenia; [2]Department of Agronomy, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, 1000 Ljubljana, Slovenia.
E-mail: octave.lacroix@ihps.si

Hops (*Humulus lupus* L.) are perennial flowering plants, cultivated for their cones used in brewing industry for their bitterness flavor and their role in preserving beer. Hops can be infected by a wide range of viroids such as hop stunt viroid (HSVd), hop latent viroid (HLVd), apple fruit crinkle viroid (AFCVd) and Citrus bark cracking viroid (CBCVd, *Cocadviroid rimocitri*). The latter is a major threat to Slovenian hop fields, with serious symptoms appearing just one year after infection, followed by plant death after 3 to 5 years. Biology of viroids was observed to be correlated with climate fluctuations. This PhD thesis aims at studying the impact of climate change on CBCVd-infected hops, modelized by two main environmental stress components: temperature (temperature increase and early/late frost) and precipitation (drought and heavy rainfall). Viroid pathogenicity in response to climate change will be analyzed through four main components: visual assessment of hop phenotype and physiological analysis of hop, analysis of hop field infection patterns over several decades, quantification of CBCVd concentration in different hop plant organs, and extraction and analysis of plant secondary metabolites. The influence of environmental stress conditions will be observed and studied in several contexts: plants placed in controlled growth chambers (in vitro, and in vivo in pot experiments), and field observations in conventional hop fields. Studying the influence of environmental stress on viroid-infected hops will provide a better understanding of viroid pathogenicity pathways and the prospects for climate-related viroid control methods.

Funded by the European Union within the Horizon Europe MSCA Doctoral Networks, Reference Number HORIZON-MSCA-2023-DN-01, Project ViroiDoc, Grant Agreement Number: 101169421. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

Poster 26 – DN3

Development of a disposable, selective, and sensitive electrochemical sensor for on-site detection of plant viroids

W.G. Liu[1], S. B. Hočevar[1], N. Tasić[1], S. Radišek[2], M. Fojta[3], A. Merkoçi[4]

[1]National Institute of Chemistry (NIC), Hajdrihova 19, 1000 Ljubljana, Slovenia; [2]Slovenian Institute of Hop Research and Brewing, Cesta Žalskega tabora 2, 3310 Žalec, Slovenia, [3]CAS – Institute of Biophysics, Veveří 97, 602 00 Brno, Czech Republic; [4]ICN2 – Nanobioelectronics & Biosensors, Baldiri Reixac 10-12, 08028 Barcelona, Spain.
E-mail: cleonlwg@gmail.com

Plant viroids are small, non-coding RNA pathogens responsible for diseases causing bark cracking, stunted growth, and reduced yields, remaining frequently undetected until late stages of infection. For example, Citrus bark cracking viroid (CBCVd) is one of them, presenting a typical threat, as early symptomless infections facilitate undetected spread, leading to substantial yield losses. To address the need for reliable field diagnostics, this project tackles the development of a powerful and disposable electrochemical sensor for on-site detection of CBCVd and/or related plant viroids. To build an efficient electrochemical detection platform, the work will begin with the selection of an optimal supporting electrode material, followed by tailored (chemical and/or electrochemical) electrode surface pretreatment to provide enhanced electrochemical reproducibility and responsiveness towards standard redox systems. In the next step, the electrode surface will be modified with signal-amplifying interface(s) based on carbon and metal nanostructures, such as carbon nanotubes, MXenes, (mixed) metal oxides, etc. These materials can be used alone or combined with various polymeric and other matrices/binders, as hybrid composites. The so prepared ((electro)catalytic) interfaces will be prudently functionalized with sequence-specific DNA or RNA capture probes and protected by antifouling layer to minimize non-specific adsorption. Different electrochemical detection modes will be examined and optimized, including amperometry, (pulse)voltammetry, and electrochemical impedance spectroscopy. Optional amplification strategies will also be explored, if needed, such as using secondary reporter probes or integrated redox mediator layers. Finally, the sensor will be evaluated in both simulated and real samples It will be adapted preferentially to screen-printed electrode formats and designed with a dual-working-electrode configuration to provide also a built-in negative control. Its robustness against potential interferents will be evaluated. This platform will offer a potentially versatile tool for routine field surveillance of viroid infections, supporting precision agriculture and sustainable crop protection.

Funded by the European Union within the Horizon Europe MSCA Doctoral Networks, Reference Number HORIZON-MSCA-2023-DN-01, Project ViroiDoc, Grant Agreement Number: 101169421. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

Poster 27 – DN4

In vivo imaging of viroid RNA and associated host factors

J. E. Lopez Ponce, L. Elvira-Gonzalez, T. Blevins, M. Heinlein

Institut de Biologie Moléculaire des Plantes, CNRS, Université de Strasbourg, 67000, Strasbourg, France.
E-mail: judith.lopez659@hotmail.com

Viroids are the smallest known plant pathogens, consisting of a circular, non-coding RNA molecule. Despite their simplicity, they can cause significant agricultural losses and pose an emerging threat to sustainable crop production due to increased international trade and climate change. Understanding how viroids interact with host plants is essential to develop new strategies for disease control.
Our project aims to visualize viroid RNA in living plant cells and to identify host factors involved in viroid trafficking. Using advanced RNA labeling techniques, including Broccoli aptamers, and dCAS-GFP, we will track the movement and localization of viroid RNA *in vivo*. These approaches will be combined with high-resolution confocal microscopy and complemented by affinity isolation or proximity labeling (TurboID) coupled to mass spectroscopy to identify host proteins that interact with the viroid RNA. Candidate proteins will be further validated by colocalization, FRET-FLIM, and reverse genetic approaches.
This technically challenging but innovative project will provide new insights into viroid biology, particularly into RNA movement and host interactions. Ultimately, our work will contribute to understanding how these minimal pathogens exploit plant systems, potentially pointing towards novel antiviral strategies.

Funded by the European Union within the Horizon Europe MSCA Doctoral Networks, Reference Number HORIZON-MSCA-2023-DN-01, Project ViroiDoc, Grant Agreement Number: 101169421. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

Poster 28 – DN5

CRISPR-based strategies for viroid detection: towards point-of-care diagnosis

Linh-Thi-Thuy Le, J.-A. Daròs

Instituto de Biología Molecular y Celular de Plantas (CSIC-Universitat Politècnica de València), 46022 Valencia, Spain.
E-mail: lethuylinh2742@gmail.com

Viroids are the smallest known infectious agents, consisting of tiny, circular, non-coding RNA molecules that infect plants and cause severe symptoms such as stunting, leaf distortion, and significant yield loss. Current diagnostic methods, such as RT-PCR, provide high accuracy but require sophisticated laboratory infrastructure, limiting their use in the field. There is a critical need for rapid, portable, and sensitive diagnostic tools to enable early detection and prevent crop losses.
CRISPR-based systems offer programmable and sequence-specific nucleic acid detection with high sensitivity and specificity. Platforms employing Cas12a, Cas13a/d, and Cas9 enable both amplification-based and amplification-free detection workflows. In particular, Cas12a and Cas9 approaches allow detection after isothermal amplification, while Cas13a/d platforms can directly detect viroid RNA without prior amplification.
These CRISPR detection systems can be integrated with point-of-care (POC) diagnostic devices, such as lateral flow strips or portable fluorescence readers, enabling results in less than one hour. The combination of CRISPR technologies with POC platforms represents a powerful step toward field-ready diagnostics in plant health management, providing a promising tool to control viroid outbreaks and safeguard agricultural productivity.

Funded by the European Union within the Horizon Europe MSCA Doctoral Networks, Reference Number HORIZON-MSCA-2023-DN-01, Project ViroiDoc, Grant Agreement Number: 101169421. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

Poster 29 – DN6

Development of novel antiviroidal strategies: towards drug discovery

T. Stojkovska[1], F. Di Serio[2], K. Kalantidis[3,4], P. Lisón[1]

[1]Instituto de Biología Molecular y Celular de Plantas (IBMCP), Consejo Superior de Investigaciones Científicas (CSIC), Universitat Politècnica de València (UPV) 46011 Valencia, Spain; [2]Institute for Sustainable Plant Protection (IPSP), National Research Council of Italy (CNR), Torino, Italy; [3]Department of Biology, University of Crete, Voutes University Campus, Heraklion, Crete, Greece; [4]Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, Heraklion, Crete, Greece.
E-mail: stojkovska_teodora@hotmail.com

Viroids are small, non-coding plant pathogens that cause severe diseases in important crops, yet no effective control strategies are available. This project develops a cell-based system for assessing novel antiviroidal drugs, aiming to identify compounds that could form the basis of viroid-control solutions. Tobacco BY2 cells will be engineered to constitutively express potato spindle tuber viroid (PSTVd) or Citrus exocortis viroid (CEVd), generating a robust model (BY2-Vd) to screen and characterize potential antiviroidal compounds. Using this platform, candidate molecules will be selected for efficacy testing from previous findings in our research group and related studies. Among them, salicylic acid plays a pivotal role in tomato basal resistance to CEVd, as demonstrated by *NahG* transgenic plants, which are unable to accumulate this hormone and consequently display extreme hypersusceptibility (López-Gresa et al., 2016). In addition, γ-aminobutyric acid (GABA), induced by benzothiadiazole (BTH) in tomato plants, enhances resistance to CEVd by reversing hypersusceptibility and activating defense genes (López Gresa et al., 2019). The TOR inhibitor AZD8055 mitigates PSTVd infection by restoring autophagy and strengthening tomato defenses (Silva-Valencia et al., 2024). Ethylene signaling has also been implicated, since reducing it can delay disease progression and alleviate ribosomal stress (Vázquez-Prol et al., 2020). Viroids also localize to translating polysomes and disrupt ribosome biogenesis, so agents that interfere with this interaction may relieve viroid-induced ribosome stress (Cottilli et al., 2019). To study the potential antiviroidal capacity of the compounds, their effects on BY2-Vd will be evaluated through viroid quantification by RT-qPCR and northern blotting, subcellular localization using fluorescence microscopy, profiling of small RNA populations by sRNA-seq, and expression of host marker genes (*PR1, NAC82*). Promising compounds will be subsequently tested *in planta*, using tomato plants infected with PSTVd or CEVd and *Nicotiana benthamiana* infected with PSTVd. These studies will further be extended to Chrysanthemum chlorotic mottle viroid (CChMVd), a member of the *Avsunviroidae* family. Overall, our work provides a framework for identifying antiviroidal drugs and lays the groundwork for practical solutions against viroid diseases.

References

Cottilli P, Belda-Palazón B, Adkar-Purushothama CR, Perreault JP, Schleiff E, Rodrigo I, Ferrando A, Lisón P. Citrus exocortis viroid causes ribosomal stress in tomato plants. Nucleic Acids Res. 2019

López-Gresa MP, Lisón P, Yenush L, Conejero V, Rodrigo I, Bellés JM. Salicylic Acid Is Involved in the Basal Resistance of Tomato Plants to Citrus Exocortis Viroid and Tomato Spotted Wilt Virus. PLoS One. 2016

López-Gresa MP, Payá C, Rodrigo I, Bellés JM, Barceló S, Hae Choi Y, Verpoorte R, Lisón P. Effect of Benzothiadiazole on the Metabolome of Tomato Plants Infected by Citrus Exocortis Viroid. Viruses. 2019

Prol FV, López-Gresa MP, Rodrigo I, Bellés JM, Lisón P. Ethylene is Involved in Symptom Development and Ribosomal Stress of Tomato Plants upon Citrus Exocortis Viroid Infection. Plants (Basel). 2020

Silva-Valencia S, Prol FV, Rodrigo I, Lisón P, Belda-Palazón B. TOR Inhibition Enhances Autophagic Flux and Immune Response in Tomato Plants Against PSTVd Infection. Physiol Plant. 2024

Funded by the European Union within the Horizon Europe MSCA Doctoral Networks, Reference Number HORIZON-MSCA-2023-DN-01-01, Project ViroiDoc, Grant Agreement Number: 101169421. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

Poster 30 – DN9

Viroid Affairs: Exploring the Hidden Network of RNA-Protein Interactions

V. Sánchez-Camargo[1*], K. Katsarou[2,3*], E. Bardani[2], L. Michailidou[2], S. Roussaki[2], H. van den Burg[1], K. Kalantidis[2,3]

[1]Molecular Plant Pathology, Swammerdam Institute for Life Sciences (SILS), University of Amsterdam, 1098 XH Amsterdam, The Netherlands;[2]Department of Biology, University of Crete, Voutes University Campus, Heraklion, Crete, Greece; [3]Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, Heraklion, Crete, Greece.
*Authors contributed equally
E-mail: michailidou.lena@gmail.com; katsarou@imbb.forth.gr

Viroids are commercially relevant plant pathogens characterized by an RNA genome that does not encode any proteins. Despite more than five decades of viroid research, only a handful of host proteins have been identified as critical for the viroid biological cycle. Viroid-binding protein 1 (VIRP1) is one of the most studied viroid binding host factors, although a few others such as RNA Polymerase II (RNA PolII), transcription factor TFIIIA-7ZF and Phloem Protein 2 (PP2), have been described.
This study aims to identify novel viroid partners by employing a recently developed method, termed Orthogonal Organic Phase Separation (OOPS), coupled with proteomics analysis. This technique involves *in vivo* UV-crosslinking of leaves, stabilizing RNA-protein interactions, followed by multiple purification steps. Finally, the isolated RNA-binding proteins (RBPs) are subjected to proteomics profiling. By comparing samples from plants infected with potato spindle tuber viroid (PSTVd) and healthy *Nicotiana benthamiana* plants, we identified 125 differentially regulated RBPs. Based on their predicted biological function, a number of these were selected as possible candidates for further functional characterization using Virus-Induced Gene silencing (VIGS), followed by PSTVd challenge. Understanding how viroids exploit host factors is essential for elucidating the molecular mechanisms underlying their pathogenicity and could pave the way for developing viroid-resistant plant varieties.

Funded by the European Union within the Horizon Europe MSCA Doctoral Networks, Reference Number HORIZON-MSCA-2023-DN-01, Project ViroiDoc, Grant Agreement Number: 101169421. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

Poster 31 – DN10

RNA Sprays - Precision tools for the modulation of host genes to control viroid infections

L. Palatinus, A. Koch

Universität Regensburg, Plant RNA Biology, Regensburg, Germany.
E-mail: Lara.palatinus@ur.de

Viroid infections, caused by the smallest known plant pathogens, pose a persistent threat to global agriculture and are becoming more frequent due to climate change and increased international trade. These non-coding circular RNAs exploit the plant host machinery, leading to severe crop yield and quality losses, with no effective chemical or biological control strategies available. The current management strategy relies on labour-intensive, unsustainable, and economically inefficient exclusion and eradication measures. To address this challenge, our research aims to explore RNA spray-induced gene modulation as a novel, non-transgenic strategy to enhance plant defense/resilience against viroid infections.

Our approach utilizes RNA interference (RNAi) and epigenetic regulation to modulate host gene expression, counteracting viroid-induced reprogramming. Our research focuses on investigating conserved host pathways that are targeted by viroids, analysing viroid-derived small RNAs (vdsRNAs) and their impact on gene regulation. We also use RNA molecules, including double-stranded RNA (dsRNA), circular antisense RNA (caRNA), circular RNA (circRNA) and messenger RNA (mRNA), to induce targeted gene silencing or activation.

Functional validation under controlled and field conditions will assess the efficacy of these formulations in suppressing viroid replication and mitigating symptoms. Through the integration of molecular, bioinformatics, and applied agricultural approaches, this research aims to establish RNA spray technology as a sustainable, precise, and field-deployable solution for viroid management. Beyond viroids, this strategy has broader implications for plant protection, offering an environmentally friendly alternative to conventional pesticides and aligning with global efforts toward sustainable agriculture.

Funded by the European Union within the Horizon Europe MSCA Doctoral Networks, Reference Number HORIZON-MSCA-2023-DN-01. Project ViroiDoc, Grant Agreement Number: 101169421. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

List of participants

Charith Raj Adkar-Purushothama, Université de Sherbrooke, Sherbrooke, Canada
charith.adkar@usherbrooke.ca

Verónica Aragonés, Instituto de Biología Molecular y Celular de Plantas (UPV-CSIC), Valencia, Spain
varagoni@ibmcp.upv.es

Marc Balanzá Garcia, Instituto de Biología Molecular y Celular de Plantas (UPV-CSIC), Valencia, Spain
marbaga1@etsiamn.upv

Eirini Bardani, University of Crete, Heraklion, Greece
ebardani@ethz.ch

Nihal Buzkan, Kahramanmaraş Sütçü Imam University, Kahramanmaraş, Türkiye
buzkan@hotmail.com

Savita Chaudhary, CSIR—Institute of Himalayan Bioresource Technology, Palampur, India
savita.ihbt20a@acsir.res.in

Michela Chiumenti, Istituto per la Protezione Sostenibile delle Piante - CNR, Bari, Italy
michela.chiumenti@cnr.it

João Francisco Parraga Ribeiro Colaço, University of Ljubljana, Ljubljana, Slovenia
kiko.114@hotmail.com

José-Antonio Daròs, Instituto de Biología Molecular y Celular de Plantas (UPV-CSIC), Valencia, Spain
jadaros@ibmcp.upv.es

Marcos de la Peña, Instituto de Biología Molecular y Celular de Plantas (UPV-CSIC), Valencia, Spain
rivero@ibmcp.upv.es

Rita Milvia de Miccolis Angelini, Università degli Studi di Bari Aldo Moro, Bari, Italy
ritamilvia.demiccolisangelini@uniba.it

Francesco Di Serio, Istituto per la Protezione Sostenibile delle Piante - CNR, Turin, Italy

francesco.diserio@cnr.it

Marcelo Eiras, Instituto Biológico, São Paulo, Brazil
marcelo.eiras@sp.gov.br

Laura Elvira Gonzalez, Institut de Biologie Moléculaire des Plantes - CNRS, Université de Strasbourg, Strasbourg, France
laura.elvira@ibmp-cnrs.unistra.fr

Francesco Faggioli, CREA-DC, Roma, Italy
francesco.faggioli@crea.gov.it

Cristina Formiglia, Istituto per la Protezione Sostenibile delle Piante – CNR and University of Turin, Turin, Italy
cristinaformiglia@cnr.it

Gnimah Eva Gnouamozi, University Hospital Heidelberg, Heidelberg, Germany
g.gnouamozi@gmail.com

Tanja Guček, Slovenian Institute for Hop Research and Brewing, Žalec, Slovenia
tanja.gucek@ihps.si

Yi Guo, Istituto per la Protezione Sostenibile delle Piante - CNR, Turin, Italy
yiguo@cnr.it

Michael H. Hagemann, University of Hohenheim, Stuttgart, Germany
michael.hagemann@uni-hohenheim.de

Imen Hamdi, Institut National de la Recherche Agronomique de Tunisie, Ariana, Tunisie
imen.hamdi@iresa.agrinet.tn

Yumeng Hao, Terrana Bioscience, Cambridge, Massachusetts, USA
yhao@terranabio.com

Manfred Heinlein, Institut de Biologie Moléculaire des Plantes – CNRS, Université de Strasbourg, Strasbourg, France
manfred.heinlein@ibmp-cnrs.unistra.fr

Jernej Jakše, University of Ljubljana, Ljubljana, Slovenia
jernej.jakse@bf.uni-lj.si

Marco Kaiser, Bioreba, Reinach, Switzerland
kaiser@bioreba.ch

Kriton Kalanditis, University of Crete and Institute of Molecular Biology and Biotechnology - FORTH, Heraklion, Greece
kriton@imbb.forth.gr

Konstantina Katsarou, University of Crete and Institute of Molecular Biology and Biotechnology - FORTH, Heraklion, Greece
katsarou@imbb.forth.gr

Christina Krönauer, Bavarian State Research Center for Agriculture, Hop Research Center, Wolnzach, Germany
christina.kroenauer@lfl.bayern.de

Nikoleta Kryovrysanaki, Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas (FORTH), Heraklion, Greece
nkrio@imbb.forth.gr

Octave Lacroix, Slovenian Institute of Hop Research and Brewing, Žalec, Slovenia
octave.lacroix@ihps.si

Shifang Li, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China
lishifang@caas.cn

Purificación Lisón, Instituto de Biología Molecular y Celular de Plantas (CSIC-Universitat Politècnica de València), Valencia, Spain
plison@ibmcp.upv.es

Judith E. Lopez Ponce, Institut de Biologie Moléculaire des Plantes - CNRS, Université de Strasbourg, Strasbourg, France
judith.lopez659@hotmail.com

Marta Luigi, CREA Centro di Ricerca Difesa e Certificazione (CREA-DC), Roma, Italy
marta.luigi@crea.gov.it

Karim Majzoub, Institut de Génétique Moléculaire de Montpellier (IGMM), Université de Montpellier (UM), Montpellier, France
karim.majzoub@igmm.cnrs.fr

Kleanthi Manousoudaki, Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas (FORTH), Heraklion, Greece
claire_manousoudaki@outlook.com

Grazia Marini, Immagina Biotechnology S.r.l, Pergine Valsugana, Italy
grazia.marini@immaginabiotech.com

Roberta Marziale, Istituto per la Protezione Sostenibile delle Piante - CNR, Bari, Italy
robertamarziale@cnr.it

Eleni Michailidou, University of Crete, Heraklion, Greece
michailidou.lena@gmail.com

Karin Moelling, Max Planck Institute for Molecular Genetics
moelling@molgen.mpg.de

Paolo Mussano, Istituto per la Protezione Sostenibile delle Piante - CNR and University of Turin, Turin, Italy
p.mussano@unito.it

Beatriz Navarro, Istituto per la Protezione Sostenibile delle Piante - CNR, Bari, Italy
beatriz.navarro@cnr.it

Barbora Opelka, University of South Bohemia, Ceske Budejovice, Czech republic
bajulle@email.cz

Lara Palatinus, Universität Regensburg, Regensburg, Germany
Lara.palatinus@ur.de

Vicente Pallás, Instituto de Biología Molecular y Celular de Plantas (UPV-CSIC), Valencia, Spain
vpallas@ibmcp.upv.es

Diego Palpacelli, Instituto de Biología Molecular y Celular de Plantas (UPV-CSIC), Valencia, Spain
dpalpac@doctor.upv.es

Josef Patzak, Hop Research Institute, Zatec, Czech Republic
patzak@chizatec.cz

Maria Isabella Prigigallo, Istituto per la Protezione Sostenibile delle Piante - CNR, Bari, Italy
mariaisabella.prigigallo@cnr.it

Sebastjan Radišek, Slovenian Institute of Hop Research and Brewing, Žalec, Slovenia
sebastjan.radisek@ihps.si

Detlev Riesner, Institute for Physical Biology, Heinrich-Heine-University, Heinrich-Heine-University, Düsseldorf, Germany
detlev.riesner@hhu.de

Nur Diyana Roslan, Malaysian Palm Oil Board, Persiaran Institusi, Kajang, Malaysia
diyana@mpob.gov.my

Luisa Rubino, Istituto per la Protezione Sostenibile delle Piante - CNR, Bari, Italy
luisa.rubino@cnr.it

Olga Rueda López, Instituto de Biología Molecular y Celular de Plantas (UPV-CSIC), Valencia, Spain
olruelo@ibmcp.upv.es

Teruo Sano, Hirosaki University, Japan
sano@hirosaki-u.ac.jp

Timo Schlemmer, University of Regensburg, Regensburg, Germany
Timo.Schlemmer@ur.de

Nadia Serale, Istituto per la Protezione Sostenibile delle Piante - CNR, Bari, Italy
nadiaserale@cnr.it

Anna Skelton, Fera Science Ltd., York, UK
anna.skelton@fera.co.uk

Dijana Škorić, University of Zagreb, Zagreb, Croatia
dijana.skoric@biol.pmf.unizg.hr

Natasa Štajner, University of Ljubljana, Ljubljana, Slovenia
natasa.stajner@bf.uni-lj.si

Teodora Stojkovska, Instituto de Biología Molecular y Celular de Plantas (UPV-CSIC), Valencia, Spain
stojkovska_teodora@hotmail.com

Petr Svoboda, Hop Research Institute, Zatec, Czech Republic

svoboda@chizatec.cz

Massimo Turina, Istituto per la Protezione Sostenibile delle Piante - CNR, URT Brescia, Italy
massimo.turina@cnr.it

Ganesan Vadamalai, Universiti Putra Malaysia, Serdang, Malaysia
ganesanv@upm.edu.my

Georgios Vidalakis, University of California, Riverside, USA
vidalg@ucr.edu

Helena Volk, University of Ljubljana, Ljubljana, Slovenia
helena.volk@bf.uni-lj.si

Hongqing Wang, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China
lishifang@caas.cn

Ying Wang, Department of Plant Pathology, University of Florida, USA
ying.wang1@ufl.edu

Tatiane Graziele Zambiassi, Universidade Federal do Paraná, Curitiba, Brazil
tzambiassi@ufpr.br

Zhixiang Zhang, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China
zhzhxiang2003@163.com

Ivan N. Zheludev, Stanford University, Stanford, USA
Ivan.zheludev@gladstone.ucsf.edu

Jelena Zindović, University of Montenegro, Podgorica, Montenegro
jelenazindovic@yahoo.com