2024 International Congress on Invertebrate Pathology and Microbial Control
&
56th Annual Meeting of the Society for Invertebrate Pathology

Vienna, July 28 – Aug. 1, 2024

TECHNISCHE UNIVERSITÄT WIEN

Table of Contents

Meeting at a Glance

Day 1	Sunday, July 28, 2024	
Room / **Time**	**Seminar Room 363 (1st floor)**	**Foyer Gusshaus**
9:00 - 17:00/ 13:00 – 17:00	**from 9:00 SIP Council Meeting**	**from 13:00 Registration Desk**
18:30 - 22:00	**Mixer (Foyer Gusshaus)**	

Day 2	Monday, July 29, 2024			
Room / **Time**	**EI 7 Lecture Hall**			
08:30 - 10:30	**Opening Ceremony** **Founders` Lecture** Honoree: Gisbert Zimmermann Lecturer: Jørgen Eilenberg			
10:30 - 11:00	Refreshment Break			
11:00 - 13:00	**Plenary Session**: Diseases of Mass-Reared Insects for Sterile Insect Technique (Chairs: Adly Abdallah, Johannes Jehle) *Rui Cardoso Pereira*: The Sterile Insect Technique: its application and challenges *Erin Schuenzel*: The application of culturing, DNA and RNA community sequencing in identifying and developing diagnostic assays for microbial pathogens in *Anastrepha ludens* mass-rearing facilities *Irene Meki*: Management of viruses in insect mass rearing facilities for the sterile insect technique: Glossina pallidipes salivary gland hypertrophy virus (GpSGHV) as an example *Sean Moore*: Challenges and solutions to viral contamination in mass rearing of codling moth and false codling moth for the sterile insect technique			
13:00 - 14:00	Lunch Break (Foyer Gusshaus) **Student Career Event (Seminar Room 363, 1st floor)**			
Room	**EI 7 Lecture hall**	**EI 8 Pötzl**	**EI 9 Hlawka**	**EI 10 Fritz Paschke**
14:00 - 16:00	**Contributed Papers** **Viruses 1**: Viral Infection, Discovery and Evolution (Chairs: Astrid Bryon, Gaelen Burke)	**Contributed Papers** **Fungi 1:** Fungal Diversity and Biocontrol Application (Chairs: Jørgen Eilenberg, Ann Hajek)	**Contributed Papers** **Microbial Control 1**: Advances in Microbial Control Agents (Chairs: Surendra Dara, Heiri Wandeler)	**Contributed Papers** **Diseases of Beneficial Invertebrates 1**: Pathogen and Symbiont: Host Interactions in aquatic Invertebrates and Bees (Chairs: Ronny van Earle, Ibtissem Ben Fekih)
16:00 - 16:30	Refreshment Break			
16:30 - 18:30	**Symposium Viruses**: Endogenized Viruses (Chair: Monique van Oers)	**Symposium Nematodes**: Past, Present & Future of Ento-mopathogenic Nematodes: 100 Years of Research (Chairs: David Shapiro-Ilan, Ivan Hiltpold)	**Contributed Papers** **Bacteria 1**: Structure and Mechanism of Action (Chairs: Yanchao Yang, Juan Luis Jurat-Fuentes)	**Symposium Microsporidia:** Current and Future Applications of Microsporidia Research (Chair: Judy Chen)
18:30 - 19:00	Break			
19:00 - 20:00		**Student Business Meeting**		
20:00 - 21:30	**Business Meeting Viruses**	**Business Meeting Bacteria**	**Business Meeting Nematodes**	**Business Meeting Microsporidia**

Day 3	Tuesday, July 30, 2024		
Room / **Time**	**EI 8 Pötzl**	**EI 9 Hlawka**	**EI 10 Fritz Paschke**
6:20 - 8:00	**5k run**		
09:00 - 11:00	**Contributed Papers** **Microbial Control 2**: Innovations in Fungal Pathogens for Pest Management (Chair: Stefan Jaronski)	**Contributed Papers** **Bacteria 2:** Resistance and Receptors (Chairs: Susana Vilchez, María Lázaro-Berenguer)	**Workshop Fungi & Nematodes**: Taxonomic Vandalism and its Implications in Invertebrate Pathology (Chairs: Patricia Stock, Chengshu Wang)
11:00 - 11:30	Refreshment Break		
11:30 - 13:30	**Symposium Microbial Control**: Microbial Marvels: Shaping the Future of Integrated Pest Management (Chair: Chad Keyser)	**Contributed Papers** **Viruses 2**: Viral Population Genetics and Ecology (Chairs: Zhihong Hu, Yang Kai)	**Contributed Papers** **Nematodes**: Advances in EPN Research for sustainable Insect Pest Management (Chairs: Ivan Hiltpold, Dana Ment)
13:30 - 14:30	Lunch Break (Foyer Gusshaus)		
14:30 - 17:30	**Excursion**		
18:00 -22:00	**BBQ (Brandauer Schlossbräu, Am Platz 5, 1130 Vienna)**		

Day 4	Wednesday July 31, 2024		
Room / **Time**	**EI 8 Pötzl**	**EI 9 Hlawka**	**EI 10 Fritz Paschke**
08:30 - 10:30	**Cross-Division Symposium Microbial Control & Nematodes**: New under the Sun - Progress in Mass Production and Formulation of Microbials (Chairs: Dana Ment, Patricia Navarro, Chad Keyser)	**Contributed Papers** **Viruses 3**: Viral covert Infections (Chairs: Wang Xi, Michael Jukes)	**Contributed Papers** **Bacteria 3**: Engineering and applied Aspects (Chairs: Luca Ruiu, Dafne Toledo)
10:30 - 11:00	Refreshment Break		
11:00 - 13:00	**Contributed Papers** **Microbial Control 3**: Integrated Pest Management and emerging Biocontrol Technologies (Chair: Jarrod Leland)	**Symposium Diseases of Beneficial Invertebrates**: Sex-distorting Parasites: Pathological and ecological Consequences (Chair: Jamie Bojko)	**Symposium Fungi**: Fungal Interaction and Management of beneficial Insects (Chairs: Nemat Keyhani, Jae Su Kim)
13:00 - 14:00	Lunch Break (Foyer Gusshaus) **JIP Editorial Board Meeting (Seminar Room 363, 1st floor)** **Student Workshop (EI 8)**		
14:00 - 16:00	**Contributed Papers** **Microsporidia**: Recent Advances in Microsporidia Research (Chairs: Jonathan Snow, Courtney MacInnis)	**Contributed Papers** **Viruses 4**: Virus-Host Interaction (Chairs: Jean-Michel Drezen, Bryony Bonning)	**Symposium Bacteria**: Physiology, Safety and Taxonomy of bacterial Pesticides (Chairs: Ben Raymond, William Moar)
16:00 - 16:30	Refreshment Break		
16:30 - 18:00	**Poster (Foyer Gusshaus)**		
18:00 - 19:30	Break		
19:30 - 21:00	**Business Meeting Fungi**	**Business Meeting Microbial Control**	**Business Meeting DBI**
	ICTV Study Group (Seminar Room 363, 1st floor)		

Day 5	Thursday August 1, 2024			
Room / **Time**	**EI 7 Lecture hall**	**EI 8 Pötzl**	**EI 9 Hlawka**	**EI 10 Fritz Paschke**
08:30 - 10:30	**Contributed Papers** **Viruses 5**: Pathogenicity, Virulence and Biological Control (Chairs: Elisabeth Huguet, Umut Toprak)	**Contributed Papers** **Fungi 2**: Fungal Interaction with Hosts and beyond (Chairs: Jürg Enkerli, Enrique Quesada-Moraga)	**Contributed Papers** **Bacteria 4**: Ecology and Regulation (Chairs: Omaththage Perera, Hannah Best)	**Contributed Papers** **Diseases of Beneficial Invertebrates 2**: Pathogen and Symbiont: Host Interactions in Invertebrate Mass Production (Chairs: Elisabeth Herniou, Christina Nielsen-LeRoux)
10:30 - 11:00	Refreshment Break			
Room	**EI 7 Lecture Hall**			
11:00 - 13:00	**Membership Meeting SIP**			
13:00 - 14:00	Lunch Break (Foyer Gusshaus) **Student Jury Committee (Seminar Room 363, 1st floor)**			
Room	**EI 7 Lecture Hall**			
14:00 - 16:00	**Pan-Division Symposium Viruses & DBI & Bacteria**: Diseases in Invertebrates for Feed and Food: Global Perspectives (Chairs: Vera Ros, Helen Hesketh, Christina Nielsen-LeRoux)			
18:30 - 0:00	**Banquet - Arcotel Wimberger (Neubaugürtel 34-36, 1070 Vienna)**			

Officers Society of Invertebrate Pathology

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Vice President	Juan Luis Jurat-Fuentes, United States of America
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SIP Office	Society for Invertebrate Pathology PO Box 930082 Verona, WI 53593, US

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Division of Nematodes

Chair Ivan Hiltpold (2023–2025)

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Chair Elect Gaelen Burke (2023–2025)

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SIP – Vienna 2024

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Helen Hesketh (Centre for Ecology and Hydrology, UK)

Ivan Hiltpold (Agroscope, Switzerland)

Johannes Jehle (Julius Kühn Institute, Germany)

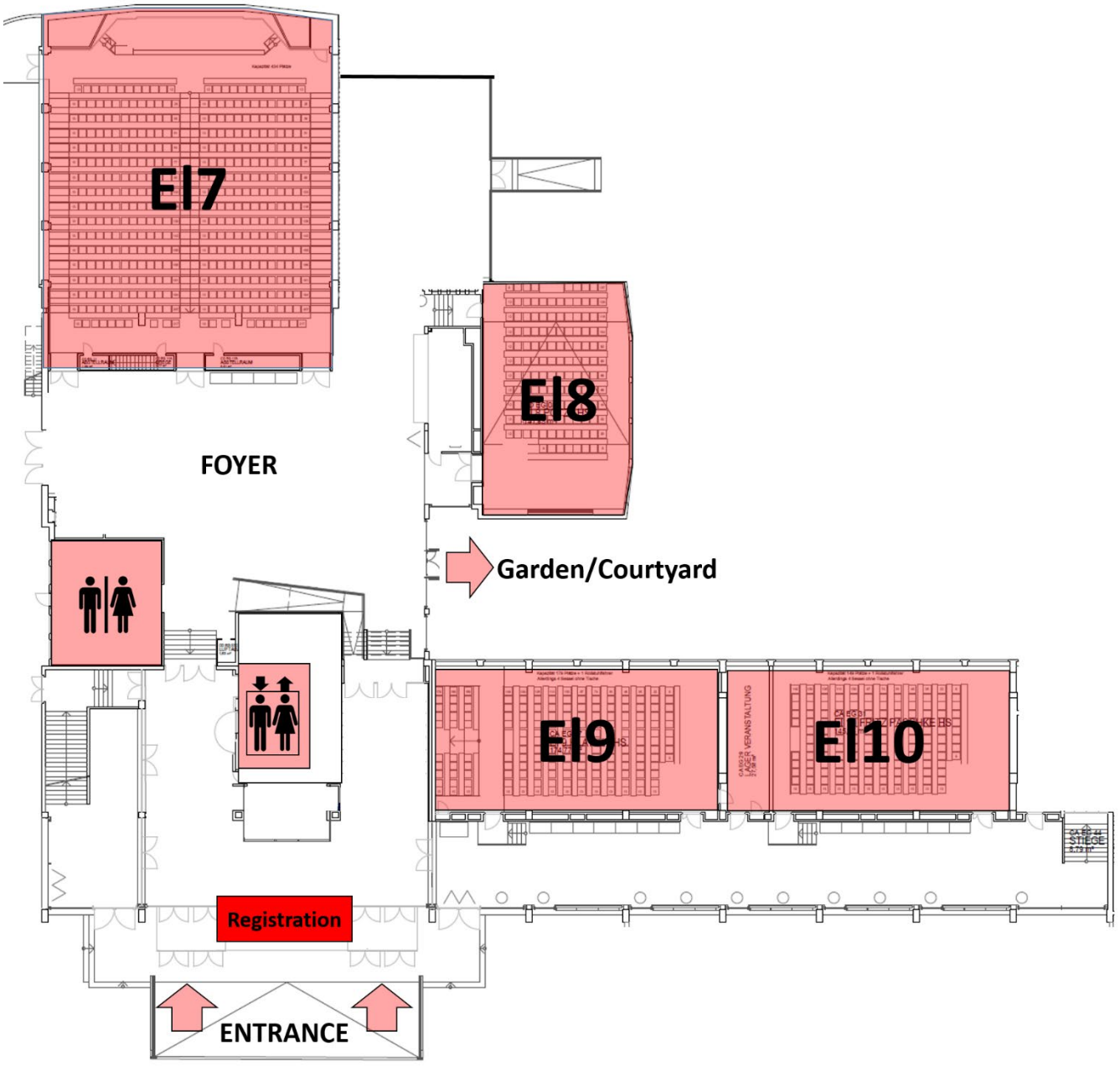
Chad Keyser (Holly Springs, USA)

Chengshu Wang (Chinese Academy of Sciences, P.R. of China)

Division Symposium Organizers

Bacteria	Ben Raymond (University of Exeter, UK), William Moar (Bayer CropScience, USA), Christina Nielsen-LeRoux (INRAE, Fance)
DBI	Jamie Bojko (Teesside University, United Kingdom), Helen Hesketh (UK Centre for Ecology & Hydrology, UK)
Fungi	Nemat Keyhani (University of Illinois at Chicago, USA), Jae Su Kim (Jeonbuk National University, Jeonju, South Korea), Chengshu Wang (Chinese Academy of Sciences, Shanghai, P.R. of China)
Microbial Control	Chad Keyser (Holly Springs, USA)
Microsporidia	YanPing (Judy) Chen (USDA-ARS Bee Research Laboratory, USA)
Nematodes	David Shapiro-Ilan (USDA-ARS, USA), Ivan Hiltpold (Agroscope, Switzerland), Patricia Stock (University of Arizona, USA), Dana Ment (Volcani Institute, Israel), Patricia Navarro (Instituto de Investigaciones Agropecuarias, Chile)
Viruses	Monique van Oers, Vera Ros (Wageningen University, The Netherlands)

The TU Vienna Conference Site

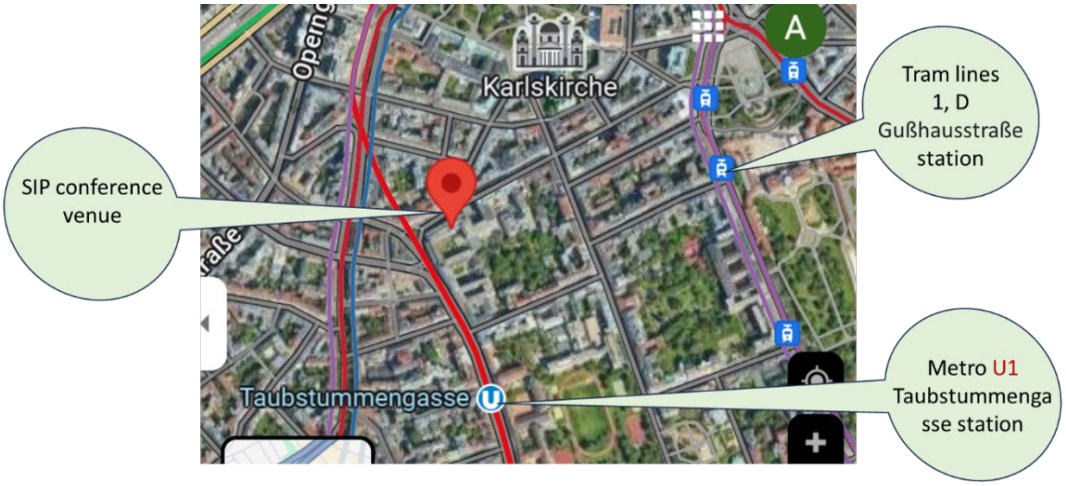


Seminar room 363

Seminar room 363 is located in the 1st floor. Follow the signs.

How to reach the conference venue with public transport

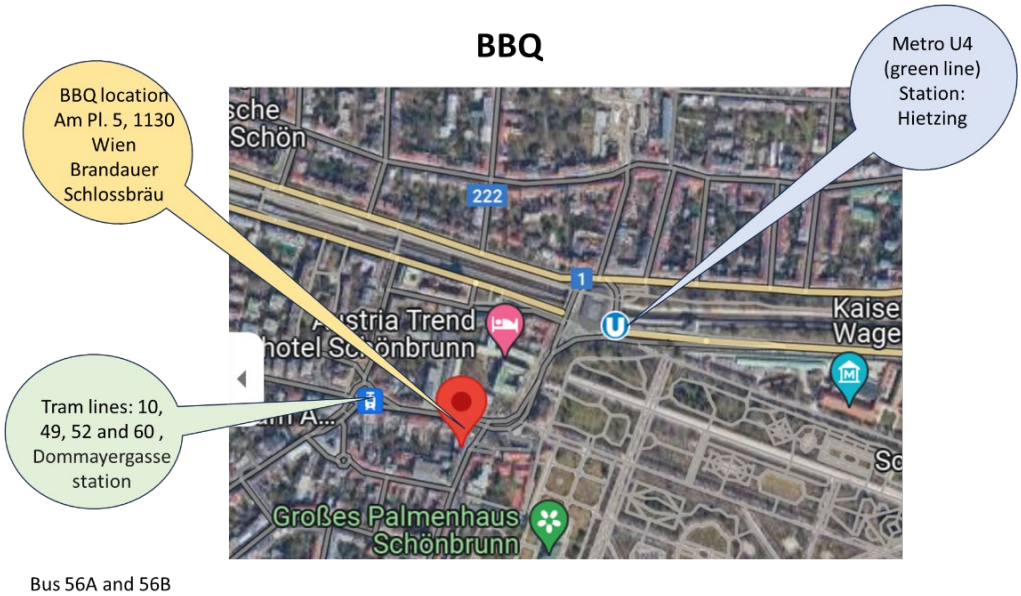
The SIP Conference 2024 will take place at Campus Gusshaus of the Technical University of Vienna, located at **Gusshausstraße 27-29, A-1040 Vienna**. Public transportation stops next to the conference venue are “Taubstummengasse” (U1) (355 m), Karlsplatz (U1, U4) (600 m), or “Gußhausstraße” (tram 1 and D) (500 m).



How to reach the BBQ location with public transport

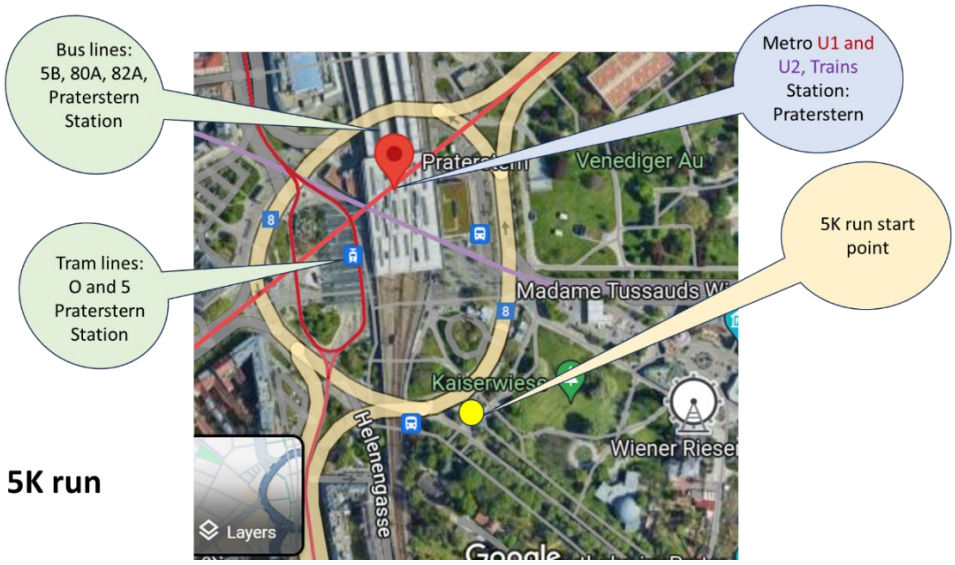
The BBQ will be held at the 'Brandauer Schlossbräu' brewery (https://www.bierig.at), **Am Platz 5, 1130 Vienna**, next to the Schönbrunn Castle. Participants of the excursions will be accompanied there by their guides. For all others, the easiest way to reach the BBQ location is using the U4 (the green Line) from “Karlsplatz” station to the “Hütteldorf” direction and leave the train at the “Hietzing” station. When leaving the station, make a left turn, cross the road, and continue walking for 2 min (~180 m). You will find the restaurant in from of you after crossing small road. If you arrive using the tram lines 10, 49, 52 or 60, exit at the station “Dommayergasse”.

You may return from the BBQ at your convenience using again the underground or tram lines.



How to reach the 5 K run location

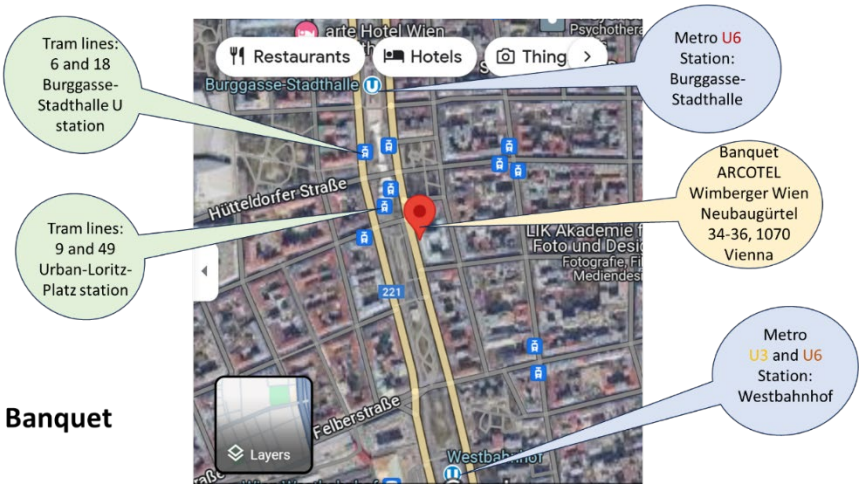
The 5K run will take place early Tuesday morning (6:30 am) in **Prater Hauptallee**, near the U1 "Praterstern" underground station. We will meet on July 30, early morning at 6:30 am in the Prater Hauptallee next to the Planetarium. The meeting point is just about 300 m from the Praterstern, a central traffic hub that can be reached by underground lines U1 and U2, trains of the S-Bahn (S1, S2, S3, S4, and S7), tram lines O and 5, and several bus lines (5B, 80A, and 82A). After the run you may return to your hotel for taking showers and having breakfast. The start of the scientific program is scheduled for 9:00 am.



How to reach the Banquet location with public transport

The Banquet will be held at the Arcotel Wimberger Hotel, **Neubaugürtel 34-36, 1070 Vienna** (https://wimberger.arcotel.com/en/) in the city center. Public transportation stops next to the Arcotel Wimberger Hotel are the underground station "Westbahnhof" (U3 and U6). Also, U6 and tram lines 6 and 18 station "Burggasse Stadthalle" are close to the hotel. Furthermore, the tram lines 9 and 49 with exit at the station "Urban-Loritz-Platz" can be used.

You may return from the Banquet at your convenience using again the underground or tram lines.



Detailed Program 2024

Presentation Guidelines

Oral Presentations

Presenting authors for oral talks and posters are indicated in ***bold/italics*** type.

Divisional and Cross-Divisional Symposia
Presentations are maximum of 25 minutes in length and 5 minutes for questions, unless otherwise specified by the chair of each symposium. Please refer to the program or contact your Symposium Chair for more information on your specific session.

Division Contributed Talks/Presentations
Presentations are a maximum of 12 minutes in length and 3 minutes for questions. Please refer to the program for specific details about your presentation.

Presentation File Submission
Please send your presentation as a .ppt file (not larger than 10 MB) not later than **24 h prior to your scheduled session** to the following address: SIP2024upload@gmail.com
Alternatively, you may upload your oral presentation file (.ppt) not later than **24 h prior to your scheduled session** at the registration desk.

For sending and uploading, please name your file with your **presentation number** (see programme) followed by the **presenter´s name** (e.g. **MC-17_Jehle.ppt** or **16-1_Herniou.ppt**)

Poster Presentations

Poster presentations will be displayed throughout the meeting and the presentation will occur during a single day and session. This will take place on Wednesday July 31, 2024 from 16:30 to 18:00 in the **Foyer Gusshaus.** Stickers will be provided, pins may not be used.
Posters shall be put up after 8:00 AM on Monday, and they must be removed by noon on Thursday or they will be discarded.

- Posters should be approximately 840 mm (2.8 ft) wide by 1190 mm (3.9 ft) tall (DIN/ISO A0). Format: Portrait NOT Landscape must be used.
- Include the poster title at the top of your poster.
- The title should be followed by a list of authors and affiliations. Please highlight the presenting author.
- Text should be readable at a distance of 120 cm. A minimum font size of 28 is recommended.
- There will be no facilities at the conference venue for poster printing or editing.

IMPORTANT

The abstracts included in this book should not be considered to be publications and should not be cited in print without the author's permission.

Participants shall **not take pictures** from projections during presentations.

[Division Initial] or **[Symposium number]-00** indicates number of **ORAL** presentation
[Division Initial]-P00 indicates number for **POSTER** presentation
-STU indicates **STUDENT** oral or poster presentation

Sunday, July 28, 2024

Executive Council Meeting
Sunday, 9:00 – 17:00
Seminar Room 363

Registration
Sunday, 13:00 – 17:00
Foyer

Welcome Mixer
Sunday, 18:30 – 22:00
Foyer

Monday, July 29, 2024

Registration Desk
Monday, 8:00 – 18:00
Foyer

Opening Ceremony and Awards Presentation
Founder's Lecture
Honoree: Gisbert Zimmermann
Lecturer: Jørgen Eilenberg
Monday, 8:30 – 10:30
Lecture Hall EI 7

Refreshment Break
Monday, 10:30 – 11:00
Foyer

PLENARY SESSION
Monday, 11:00 – 13:00
Lecture Hall EI 7

Diseases of Mass-Reared Insects for Sterile Insect Technique
Chairs: Adly Abd-Alla and Johannes Jehle

PLENARY SYMPOSIUM. Monday, 11:00 **8-1**

The Sterile Insect Technique: its application and challenges

Rui Cardoso Pereira

PLENARY SYMPOSIUM. Monday, 11:30 **8-2**

The application of culturing, DNA and RNA community sequencing in identifying and developing diagnostic assays for microbial pathogens in *Anastrepha ludens* mass-rearing facilities

***Erin Schuenzel**, Don Vacek, Roxanne Farris*

PLENARY SYMPOSIUM. Monday, 12:00 **8-3**

Management of viruses in insect mass rearing facilities for the sterile insect technique: Glossina pallidipes salivary gland hypertrophy virus (GpSGHV) as an example

***Irene Kasindi Meki**, Adly M. M. Abd-Alla*

PLENARY SYMPOSIUM. Monday, 12:30 **8-4**

Challenges and solutions to viral contamination in mass rearing of codling moth and false codling moth for the sterile insect technique

***Sean Moore**, Michael Jukes, Clarissa Mouton, Theunis Lombard, Mathew Goddard, Martin Hill, Petrus Iita, David Taylor, Siviwe Tole, Caroline Knox, Daleen Stenekamp*

Student Career Event
Monday, 13:00 – 14:00
Seminar Room 363

Lunch
Monday, 13:00 – 14:00
Foyer

Contributed Papers Viruses 1
Monday, 14:00 – 16:00
Lecture Hall EI 7

Viral Infection, Discovery and Evolution
Chairs: Astrid Byron, Gaelen Burke

EARLY CAREER AWARD Monday, 14:00

Introduction

EARLY CAREER AWARD PRESENTATION. Monday14:05 **V-1**

An ancient and conserved viral entry mechanism mediated by PIFs

***Xi Wang**, Just M. Vlak, Manli Wang, Zhihong Hu*

CONTRIBUTED PAPERS. Monday, 14:30 **V-2-STU**

The "nudist" viruses from prehistoric times: Data-driven discovery of novel nudiviruses from ectoparasitic insects prompts a taxonomic and evolutionary re-evaluation within the family *Nudiviridae*

***Jirka Manuel Petersen**, Amy Burgess, Monique M. van Oers, Elisabeth Herniou, Jamie Bojko*

CONTRIBUTED PAPERS. Monday, 14:45 **V-3-STU**

The microbiome of continuous Asian citrus psyllid cell lines

***Emily Vu**, Ke Wu, Saptarshi Ghosh, Bryony Bonning*

CONTRIBUTED PAPERS. Monday, 15:00 **V-4-STU**

The isolation, identification, and characterisation of a novel *Alphabaculovirus* isolated from *Serrodes partita*

***Tahnee Bennett**, Tapiwa Mushore, Martin Hill, Sean Moore, Michael Jukes, Caroline Knox*

CONTRIBUTED PAPERS. Monday, 15:15 **V-5**

Untangling the strings of the puppet master: investigating the role of biogenic amine signaling cascades in AcMNPV-infected *Spodoptera exigua* caterpillars

***Astrid Bryon**, Simone Nordstrand Gasque, Alexander Haverkamp, Vera I.D. Ros*

CONTRIBUTED PAPERS. Monday, 15:30 **V-6**

AcMNPV P74 is cleaved at R325 and R334 by proteinases of both OB and BBMV to expose a potential fusion peptide for oral infection

*Zhuorui Li, Nan Zhang, Tao Zhang, Zhiying Wang, Jiang Li, Manli Wang, Zhihong Hu, **Xi Wang***

CONTRIBUTED PAPERS. Monday, 15:45 **V-7**

Characterization of proteins packaged into *Venturia canescens* Virus-Like Particles and their transcriptional control

*Corinne M. Stouthamer, Ange Lorenzi, **Gaelen R. Burke***

Contributed Papers Fungi 1
Monday 14:00 – 16:00
Plötzl EI 8

Fungal Diversity and Biocontrol Application
Chairs: Jørgen Eilenberg, Ann Hajek

CONTRIBUTED PAPERS. Monday, 14:00 **F-1**

***Strongwellsea*, a specialist genus of insect pathogenic fungi, shows remarkable species diversity**

***Jørgen Eilenberg**, Verner Michelsen, Annette Bruun Jensen, Richard A. Humber*

CONTRIBUTED PAPERS. Monday, 14:15 **F-2**

Diversity and impact of fungal pathogens infecting the spotted lanternfly, *Lycorma delicatula*

*Ann Hajek**, Eric Clifton***

CONTRIBUTED PAPERS. Monday, 14:30 **F-3-STU**

The entomopathogenic genus *Beauveria* represents the predominant fungal pathogen among the adult *Popillia japonica* population in Europe

Noëmi Küng, *Denise Baur, Klaus Schläppi, Franco Widmer, Jürg Enkerli*

CONTRIBUTED PAPERS. Monday, 14:45 **F-4**

Exploration of intraspecific lineages and genetic diversity of *Beauveria pseudobassiana*

Chiara Pedrazzini*, Stephen A. Rehner, Fiona Stewart-Smith, Sara Boschi, Franco Widmer, Jürg Enkerli*

CONTRIBUTED PAPERS. Monday, 15:00 **F-5-STU**

Microbiome of North American Ash for Biocontrol of Emerald Ash Borer

Claire Yager*, Judith Mogouong, Kathryn E. Bushley*

CONTRIBUTED PAPERS. Monday, 15:15 **F-6**

Fungal parasites of nematode eggs for biocontrol of the soybean cyst nematode

Emily Green, Dong-gyu Kim, Deepak Haarith, Nin Knight, Senyu Chen, ***Kathryn E. Bushley***

CONTRIBUTED PAPERS. Monday, 15:30 **F-7**

Production and formulation of nematopathogenic fungi for control of cyst nematodes

Maximilian Paluch, Tanja Seib, ***Dietrich Stephan***

CONTRIBUTED PAPERS. Monday, 15:45 **F-8**

Soil treatments with *Metarhizium brunneum* Petch. (Ascomycota: Hypocreales) for the control of the olive fly *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) can promote olive tree growth

Antonia Romero-Conde, Meelad Yousef-Yousef, Sid Ali Benazzeddine, Andres Sandoval-Lozano, Enrique Quesada-Moraga, ***Inmaculada Garrido-Jurado***

Contributed Papers Microbial Control 1

Monday, 14:00 – 16:00
Hlawka EI9

Advances in Microbial Control Agents
Chairs: Surendra Dara, Heiri Wandeler

CONTRIBUTED PAPERS. Monday, 14:00 **MC-1**

Opportunities and challenges for microbial control of arthropod pests on the US West Coast

Surendra K. Dara

CONTRIBUTED PAPERS. Monday, 14:15 **MC-2**

Development, characterization, formulation, and use of bioinsecticides based on Baculovirus and *Bacillus thuringiensis* in Brazil.

Fernando Valicente*, Jean Pinho, Frederick Mendes, Tatiane Melo*

CONTRIBUTED PAPERS. Monday, 14:30 **MC-3**

Use of baculoviruses into conventional agriculture in the United States

Scott Ludwig

CONTRIBUTED PAPERS. Monday, 14:45 **MC-4-STU**

Surviving a baculovirus infection: impact on life history traits and potential resistance development in the fall armyworm *Spodoptera frugiperda*

Ahmed G. Hussain*, Renée A.H. van Schaijk, Emily Burdfield-Steel, Astrid T. Groot, Vera I.D. Ros*

CONTRIBUTED PAPERS. Monday, 15:00 **MC-5**

A novel Cydia pomonella granulovirus (CpGV) isolate overcomes type II resistance in codling moth

Shili Yang, ***Sarah Biganski****, Jiangbin Fan, Jörg T. Wennmann, Johannes A. Jehle*

CONTRIBUTED PAPERS. Monday, 15:15 **MC-6-STU**

Efficacy of a spray-dried formulation of Bacillus thuringiensis subsp. kurstaki (strain LIP) produced on a wheat bran-based complex medium

Rayan Nasreddine*, Gül Ayyildiz, Zhanerke Amangeldi, Asime Filiz Çalıskan Keçe, Zeynep Yurtkuran-Çeterez, Dietrich Stephan*

CONTRIBUTED PAPERS. Monday, 15:30 **MC-7-STU**

Endophyte *Beauveria bassiana* (Balsamo) Vuill. modifies cotton aphid responses: RNAseq analysis of *Aphis gossypii* Glover in response to feeding on endophytically colonized plants

Maria Cuenca-Medina*, J.V. Die, A. Pérez-Rial, E. Quesada-Moraga, N. González-Mas*

CONTRIBUTED PAPERS. Monday, 15:45 **MC-8**

A mycoviral infection drives ecological fitness of the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuill.

Fatima Ruedo Maillo, *I Garrido-Jurado, I Kotta-Loizou, E Quesada-Moraga*

Contributed Papers DBI 1

Monday, 14:00 – 16:00
Fritz Paschke EI 10

Pathogen and Symbiont: Host Interactions in aquatic Invertebrates and Bees
Chairs: Ronny van Aerle, Ibtissem Ben Fekih

CONTRIBUTED PAPERS. Monday, 14:00 **DBI-1**

Exploring the Pathobiome of White Faeces Syndrome in *Penaeus vannamei*

David Bass, Chantelle Hooper, K. P. Jithendran, K. Vinay Kumar, Subhendu Kumar Otta, P. Ezhil Praveena, R. Saraswathi, M.S. Shekhar, Grant D. Stentiford, ***Ronny van Aerle***

CONTRIBUTED PAPERS. Monday, 14:15 **DBI-2-STU**

Primed for success: Can immune priming be utilised in the Pacific oyster to improve resistance against *Vibrio aestuarianus*?

Sarah Woodsford*, Irene Cano, Frederico Batista, Ronny van Aerle, Aaron Jeffries, Robert Ellis, Adele Cobb, Samuel Melrose, Andrew Joseph, Eduarda Santos*

CONTRIBUTED PAPERS. Monday, 14:30 **DBI-3-STU**

Novel cell types and functions of *Crassostrea gigas* immune cells revealed by a comprehensive single cell transcriptomics and cytology atlas

Sébastien de la Forest Divonne*, Juliette Pouzadoux, Guillaume Mitta, Delphine Destoumieux-Garzon, Benjamin Gourbal, Guillaume M. Charrière, Emmanuel Vignal*

CONTRIBUTED PAPERS. Monday, 14:45 **DBI-4**

Growth of multiple strains of *Wolbachia* in the *Apis mellifera* cell line AME-711

Lesley Bell-Sakyi*, Jing Jing Khoo, Catherine Hartley, Christine Reitmeyer, Benjamin Makepeace, Michael Goblirsch, Luke Alphey*

CONTRIBUTED PAPERS. Monday, 15:00 **DBI-5**

The first isolation of a lactic acid bacterium (*Apilactobacillus kunkeei*) from honey bee (*Apis mellifera anatoliaca*) honey stomach and identification of its potential probiotic feature

***Mehtap Usta**, Kübra Zengin, Serhat Solmaz, Samet Okuyan, Remziye Nalçacıoğlu, Zihni Demirbağ*

CONTRIBUTED PAPERS. Monday, 15:15 **DBI-6**

CBPV exploits host AMPs to alter gut microbiota composition for viral infection

Yanchun Deng

CONTRIBUTED PAPERS. Monday, 15:30 **DBI-7**

Occurrence of honey bee pathogens in Vespa orientalis

***Karen Power**, Rebecca Leandri, Giovanni Cilia, Ernesto Ragusa, Gionata De Vico, Paola Maiolino*

CONTRIBUTED PAPERS. Monday, 15:45 **DBI-8**

***Enterococcaceae* facilitates the proliferation of CSBV in honeybee by affecting metabolic disorder**

Chunsheng Hou

Refreshment Break

Monday, 16:00 – 16:30
Foyer

Viruses Division Symposium

Monday, 16:30 – 18:30
Lecture Hall EI 7

Endogenized Viruses
Chair: Monique van Oers

VIRUSES DIVISION SYMPOSIUM. Monday, 16:30 **10-1**

The diversity and impact of endogenous viral elements in insects

Clement Gilbert

VIRUSES DIVISION SYMPOSIUM. Monday, 17:30 **10-3**

Endogenous Viral Elements in arboviral vectors: from discovery to functions

*Laila Gasmi, Davide Sogliani, Claudia Alfaro, Hugo Perdomo, Ayda Khorramnejad, Stefano Quaranta, Nfamara Cham, Alejandro Nabor Lozada-Chávez, **Mariangela Bonizzoni***

VIRUSES DIVISION SYMPOSIUM. Monday, 18:00 **10-4**

Endogenized nudiviruses in parasitic wasps and their fate in caterpillar hosts

***Elisabeth Huguet**, Thibaut Josse, Alexandra Cerqueira de Araujo, Matthieu Leobold, Camille Heisserer, Karine Musset, Annie Bézier, Germain Chevignon, Georges Périquet, Jean-Michel Drezen*

VIRUSES DIVISION SYMPOSIUM. Monday, 17:00 **10-2**

***Filamentoviridae*, an ancient family of DNA viruses influencing both the short- and long-term evolution in host-parasitoid systems**

***Julien Varaldi**, Benjamin Guinet, Matthieu Leobold, Elisabeth Herniou, Jean-Michel Drezen, Annie Bézier, Bastien Boussau, Jonathan Vogel, Ralph Peters, Jan Hrcek, Matt Buffington*

Nematodes Division Symposium

Monday, 16:30 – 18:30
Plötzl EI 8

Past, Present and Future of Entomopathogenic Nematodes: 100 Years of Research
Chairs:David Shapiro-Ilan,Ivan Hiltpold

NEMATODES DIVISION SYMPOSIUM. Monday, 16:30 **12-1**

Past, Present and Future of *Xenorhabdus* and *Photorhabdus* bacteria

*Selcuk Hazir, **David Shapiro-Ilan***

NEMATODES DIVISION SYMPOSIUM. Monday, 16:54 **12-2**

How molecules and molecular tools have intertwined in the discovery and life history of entomopathogenic nematodes

S. Patricia Stock

NEMATODES DIVISION SYMPOSIUM. Monday, 17:18 **12-3**

Bringing EPNs to higher levels – the journey for off-ground application

*Dana Ment**, Jayashree Ramakrishnan, Liora Salame, Itamar Glaze***

NEMATODES DIVISION SYMPOSIUM. Monday, 17:42 **12-4**

Advances in Formulation and Application Technology for Entomopathogenic Nematodes

David Shapiro-Ilan

NEMATODES DIVISION SYMPOSIUM. Monday, 18:06 **12-5**

Chemical ecology of entomopathogenic nematodes: back to the future

***Ivan Hiltpold**, Sergio Rasmann, Jared, G. Ali*

Contributed Papers Bacteria 1

Monday 16:30 – 18:30
Hlawka EI 9

Structure and Mechanism of Action
Chairs: Yanchao Yang, Juan Luis Jurat-Fuentes

CONTRIBUTED PAPERS. Monday, 16:30 **B-1-STU**

Characterization of Cry1Aa binding through protein mutants in two lepidopteran pests

***Dafne Toledo**, Yolanda Bel, Baltasar Escriche*

CONTRIBUTED PAPERS. Monday, 16:45 **B-2**

VIP3Cb1 structural & functional studies: Insecticidal toxin from *Paenibacillus* spp. effective for controlling Lepidopteran insect pests in crops

*Tommi White, Meiying Zheng, Timothy Rydel, Michael Rau, William Moar, Todd Ciche, **David Bowen**, Lucas Mckinnon, James Fitzpatrick, Adam Cutts, Colin Berry*

CONTRIBUTED PAPERS. Monday, 17:00 **B-3**

Analysis of Synergism between Extracellular Polysaccharide from *Bacillus thuringensis* and Insecticidal Protein

*Meiling Wang, Bai Xue, Tianjiao Ma, Zeyu Wang, Changlong Shu, Lili Geng, **Jie Zhang***

CONTRIBUTED PAPERS. Monday, 17:15 **B-4-STU**

Receptor Interactions of Vip3Aa Protoxin and Activated-Protein Structural Conformations in *Spodoptera exigua*

***María Lázaro-Berenguer**, Juan Ferré, Patricia Hernández-Martínez*

CONTRIBUTED PAPERS. Monday, 17:30 **B-5**

***Bacillus thuringiensis* Vip3Aa structural changes upon proteolytic activation trigger receptor binding necessary for insect toxicity**

Oscar Infante, Isabel Gómez, Angel E. Pélaez, Luis A. Verduzco-Rosas, Rosalina García-Suárez, Zeyu Wang, Jie Zhang, Alejandra Bravo, ***Mario Soberón***

CONTRIBUTED PAPERS. Monday, 17:45 **B-6-STU**

Interactions of vegetative insecticidal proteins with target membranes

Adam Cutts*, Paola Borri, William Moar, Tommi White, Colin Berry*

CONTRIBUTED PAPERS. Monday, 18:00 **B-7**

The folding-cane model to explain major conformational changes of *Bacillus thuringiensis* Cry1Ab required for membrane insertion and toxicity

Sabino Pacheco, Jorge Sánchez, Isabel Gómez, Blanca García, Mario Soberón, ***Alejandra Bravo***

CONTRIBUTED PAPERS. Monday, 18:30 **B-8-STU**

The first pore structure of the independent and insecticidal Bacterial Exotoxin B protein Vpb4

Raymond Wirawan*, Bradley Spicer, Oliver Castell, Charles Bayly-Jones, Chris Lupton, David Jamieson, Hannah Baird, Dafydd Jones, Lainey Williamson, Husam Sabah Auhim, Hannah Best, Hari Venugopal, Colin Berry, Michelle Dunstone*

Microsporidia Division Symposium
Monday, 16:30 – 18:30
Fritz Paschke EI 10

Current and Future Applications of Microsporidia Research
Chair: Judy Chen

MICROSPORIDIA DIVISION SYMPOSIUM. Monday, 16:30 **9-1**

Microsporidian Taxonomy: Things to Tie Up

Jamie Bojko

MICROSPORIDIA DIVISION SYMPOSIUM. Monday, 17:00 **9-2**

Effects of *Vairimorpha* (*Nosema*) *ceranae* and *Lotmaria passim* infections on honey bee behaviour and physiology

Courtney MacInnis, *Lien Luong, Steve Pernal*

MICROSPORIDIA DIVISION SYMPOSIUM. Monday, 17:30 **9-3**

Microsporidian Genomes: Tales of polyploidy, rearrangements, and recombination

Amjad Khalaf, *Kamil Jaron, Mark Blaxter, Mara Lawniczak*

MICROSPORIDIA DIVISION SYMPOSIUM. Monday, 18:00 **9-4**

The molecular mechanisms underlying the vertical transmission of *Nosema bombycis* in silkworms provide an opportunity for molecular breeding

Chunxia Wang, Bin Yu, Yongzhi Kong, Zishen Tang, Tongyu Luo, ***Tian Li***

Student Business Meeting
Monday, 19:00 – 20:00
Plötzl EI 8

Viruses Division Business Meeting
Monday, 20:00 – 21:30
Lecture Hall EI 7

Bacteria Division Business Meeting
Monday, 20:00 – 21:30
Plötzl EI 8

Nematodes Division Business Meeting
Monday, 20:00 – 21:30
Hlawka EI 9

Microsporidia Division Business Meeting
Monday, 20:00 – 21:30
Fritz Paschke EI 10

Tuesday,July 30, 2024

5K Run/Walk at Prater Stern
Tuesday, 6:20 – 8:00
Transportation by public transportation
Runners meet at starting point at 6:20 (Start run 6:30)

Registration Desk
Tuesday, 8:00 – 14:00
Foyer

Contributed Papers Microbial Control 2
Tuesday, 9:00 – 10:30
Plötzl EI 8

Innovations in Fungal Pathogens for Pest Management
Chair: Stefan Jaronski

CONTRIBUTED PAPERS. Tuesday, 9:00 **MC-9**

Potential of entomopathogenic nematodes and entomopathogenic fungi against coconut rhinoceros beetle in Hawaii

Zhiqiang Cheng

CONTRIBUTED PAPERS. Tuesday, 9:15 **MC-10**

Innovative solutions to control *Asproparthenis punctiventris* with the entomopathogenic fungus *Metarhizium brunneum*

Maria Zottele*, Martina Dokal, Jürg Enkerli, Hermann Strasser*

CONTRIBUTED PAPERS. Tuesday, 9:30 **MC-11-STU**

Potential of endophytic *Beauveria bassiana* for the management of *Coraebus undatus* (Coleoptera: Buprestidae) in cork oak forests

Morda Walaa*, Lentini Andrea, Mannu Roberto, Olivieri Maurizio, Luca Ruiu*

CONTRIBUTED PAPERS. Tuesday, 9:45 **MC-12**

Comparative genomics study on commercial strains of *Beauveria bassiana*: how genetic variability affects insecticidal activity

Gabriele Moro*, Maria Giovanna Marche*

CONTRIBUTED PAPERS. Tuesday, 10:00 **MC-13**

Strategising the all-season usage of *Hirsutella thompsonii* [ICAR–NBAIR–MF(Ag)66] to manage the broad mite in mulberry

Sreerama Kumar Prakya*, Nanjundaiah Sheela, Chikkalingaiah Bindushree*

CONTRIBUTED PAPERS. Tuesday, 10:15 **MC-14**

Implementing indirect effects of entomopathogenic fungi in biocontrol of spider mites

Stine K. Jacobsen, ***Nicolai V. Meyling***

Contributed Papers Bacteria 2

Tuesday, 9:00 – 11:00
Hlawka EI 9

Resistance and Receptors
Chairs: Susana Vilchez, María Lázaro-Berenguer

CONTRIBUTED PAPERS. Tuesday, 9:00 **B-9**

Development and characterization of western corn rootworm, *Diabrotica virgifera virgifera* LeConte Resistant to Mpp75Aa1.1 from *Brevibacillus laterosporus* and Vpb4Da2 from *Bacillus thuringiensis*

***William Moar**, Chitvan Khajuria, Kaylee Miller, Michael Pleau, Brent Werner, Yong Yin, Paula Price, Juan Ferré, Graham Head*

CONTRIBUTED PAPERS. Tuesday, 9:15 **B-10**

Characterization of a *Caenorhabditis elegans* strain highly resistant to Cry14A family proteins

*YouMie Kim, Thanh-Thanh Nguyen, Takao Ishidate, Daniel Durning, Ozkan Aydemir, Craig Mello, Yan Hu, **Raffi Aroian***

CONTRIBUTED PAPERS. Tuesday, 9:30 **B-11 STU**

Lack of tolerance development following sublethal Cry1 protein exposure in *Spodoptera exigua* (Hübner)

***Sandy Valdiviezo-Orellana**, Patricia Hernández-Martínez, Baltasar Escriche*

CONTRIBUTED PAPERS. Tuesday, 9:45 **B-12-STU**

Knockdown of *Egfr* and *Stmn4* by using an efficient oral feeding RNAi system increases the susceptibility of *Spodoptera frugiperda* larvae to Cry1F protein

***Yanchao Yang**, Zeyu Wang, Jie Zhang*

CONTRIBUTED PAPERS. Tuesday, 10:00 **B-13**

Impacts of gene introgression from invasive *Helicoverpa armigera* into native *Helicoverpa zea* on the efficacy of transgenic crops in the USA

*Marissa I. Nufer, Brad S. Coates, Craig A. Abel, Patrick O´Neill, Morgan McCraken, Devendra Jain, Calvin A. Pierce, James Glover, Tyler Towles, Gadi V. P. Reddy, **Omaththage P. Perera***

CONTRIBUTED PAPERS. Tuesday, 10:15 **B-14**

Identifying resistance alleles to Bt corn in European corn borer with multiplexed targeted sequencing

*Yasmine Farhan, Yamikani Ngona, Tom Ruttink, Peter Tandy, Kurt Lamour, Jocelyn Smith, Andrew Michel, **Juan Luis Jurat-Fuentes***

CONTRIBUTED PAPERS. Tuesday, 10:30 **B-15**

New paralogs of the *Heliothis virescens* ABCC2 transporter as potential receptors for Bt Cry1A proteins

*Daniel Pinos, Anabel Millán-Leiva, Juan Ferré, **Patricia Hernández-Martínez***

CONTRIBUTED PAPERS. Tuesday, 10:45 **B-16**

Genetics of insect resistance to Vip3Aa

*Zhenxing Liu, Chongyu Liao, Luming Zou, Minghui Jin, Yinxue Shan, Yan Peng, Huihui Zhang, Hui Yao, Lei Zhang, Peng Wang, Zhuangzhuang Liu, Na Wang, Yutao Xiao, Anjing Li, Kaiyu Liu, Wenhui Wang, Yudong Quan, Kongming Wu, Bruce Tabashnik, **David Heckel***

Panel Discussion Workshop Nematodes and Fungi Divisions

Tuesday, 9:00 – 11:00
Fritz Paschke EI 10

Taxonomic Vandalism and its Implications in Invertebrate Pathology
Chairs: Patricia Stock, Chengshu Wang

WORKSHOP NEMATODES & FUNGI DIVIS. Tuesday, 9:00

Introduction

Patricia Stock, Chengshu Wang

WORKSHOP NEMATODES & FUNGI DIVIS. Tuesday, 9:10 **18-1**

Back to Basics: Reconciling Conflicting Taxonomic Practices

Richard A. Humber

WORKSHOP NEMATODES & FUNGI DIVIS. Tuesday, 9:22 **18-2**

Understanding the environmental context of entomopathogenic fungi: Fungal community dynamics surrounding mycosed invertebrates and isolation of new entomopathogenic fungal species

*Ross Joseph, Abolfazl Masoudi, **Nemat Oliver Keyhan**i*

WORKSHOP NEMATODES & FUNGI DIVIS. Tuesday, 9:34 **18-3**

A brief history of *Beauveria bassiana and Metarhizium anisopliae*: Resolving historical problems and anticipating future challenges

***Kathryn E. Bushley**, Stephen A. Rehner*

WORKSHOP NEMATODES & FUNGI DIVIS. Tuesday, 9:46 **18-4**

Addressing Taxonomic Vandalism in Entomothogenic Nematodes: Reflections on Past Successes and Future Challenges

Vladimír Půža

WORKSHOP NEMATODES & FUNGI DIVIS. Tuesday, 9:58 **18-5**

Current methods for the taxonomy and systematics of the entomopathogenic bacterial genera *Photorhabdus and Xenorhabdus*

Ricardo Machado

WORKSHOP NEMATODES & FUNGI DIVIS. Tuesday, 10:10

Discussion, Q&A with Panelist and audience

Refreshment Break

Tuesday, 11:00 – 11:30
Foyer

Microbial Control Division Symposium

Tuesday, 11:30 – 13:00
Plötzl EI 8

Microbial Marvels: Shaping the Future of Integrated Pest Management
Chair: Chad Keyser

MC DIVISION SYMPOSIUM. Tuesday, 11:30 **15-1**

Getting more for your money. Can we exploit interactions of microbial and chemical pesticides for increased pest control?

***Eleanor L. Dearlove**, David Chandler, Steve Edgington, Shaun D. Berry, Gareth Martin, Claus Svendsen, Helen Hesketh*

MC DIVISION SYMPOSIUM. Tuesday, 12:00 **15-2**

Testing Microbial Pest Control Products as components of IPM programs: considerations for appropriate trial design

Edith Ladurner

MC DIVISION SYMPOSIUM. Tuesday, 12:30 **15-3**

Future position of Biocontrol in Integrated Pest Management

Roma Gwynn

Contributed Papers Viruses 2

Tuesday, 11:30 – 13:30
Hlawka EI 9

Viral Population Genetics and Ecology
Chairs: Zhihong Hu, Yang Kai

CONTRIBUTED PAPERS. Tuesday, 11:30 **V-8**

Genetic diversity of RNA viruses infecting invertebrate pests of rice

Haoran Wang, Shufen Chao, Qing Yan, Shu Zhang, Guoqing Chen, ***Guozhong Feng***

CONTRIBUTED PAPERS. Tuesday, 11:45 **V-9**

The haplotypic structure in baculovirus isolates deciphered by SNV linkage and machine learning

Jörg T. Wennmann*, Jens Keilwagen, Mudasir Gani, Ahmed G. Hussain, Vera I.D. Ros, Johannes A. Jehle*

CONTRIBUTED PAPERS. Tuesday, 12:00 **V-10**

A *cis*-acting negative regulator of baculovirus spread catalyzes natural selection of deletion genotypes in cell culture

Just M. Vlak*, Amaya Serrano, Delia Munoz, Corinne Geertsema, Primitivo Caballero, Gorben Pijlman*

CONTRIBUTED PAPERS. Tuesday, 12:15 **V-11**

Gene flow analysis of Cnaphalocrocis medinalis granulovirus in southern China*Yun Gu, Baoding Chen, Junxiong Yang, Yachao Zuo, Meijin Yuan,* ***Kai Yang***

CONTRIBUTED PAPERS. Tuesday, 12:30 **V-12**

Erv family proteins are the relics of pre-cellular life

Huanyu Zhang*, Wenhua Kuang, Shu Liu, Qiuhui Hou, Xinyu Zhang, Jiang Li, Zhihong Hu, Manli Wang.*

CONTRIBUTED PAPERS. Tuesday, 12:45 **V-13**

Construction of an AcMNPV minigenome by synthetic biology

Yijia Guo, Hengrui Hu, Han Xiao, Xi Wang, Jiang Li, Manli Wang, ***Zhihong Hu***

CONTRIBUTED PAPERS. Tuesday, 13:00 **V-14**

Insights into the Cell Fusing Agent Virus infection dynamics and its regulation by *Aedes albopictus* non retroviral endogenous viral elements

Laila Gasmi*, Davide Sogliani, Claudia Alfaro, Claudio Casali, Marco Biggiogera, Mariangela Bonizzoni*

CONTRIBUTED PAPERS. Tuesday, 13:15 **V-15**

Assessment of insect specificity and pathogenicity of RNA and DNA viruses for GMO safety evaluation

Just M. Vlak

Contributed Papers Nematodes

Tuesday, 11:30 – 12:15
Fritz Paschke EI 10

Advances in EPN Research for sustainable Insect Pest Management
Chairs: Ivan Hiltpold, Dana Ment

CONTRIBUTED PAPERS. Tuesday, 11:30 **N-1**

A safe and effective control method against the fall armyworm with entomopathogenic nematodes

Patrick Fallet*, Didace Bazagwira, Carlos Bustos-Segura, Joelle Kajuga, Stefan Toepfer, Ted C.J. Turlings*

CONTRIBUTED PAPERS. Tuesday, 11:45 **N-2**

Increasing the efficacy of Steinernema australe after fourteen generations by using volatile root signals of blueberry plants

Patricia D. Navarro*, Almendra J. Monje, Valentina Gallegos*

CONTRIBUTED PAPERS. Tuesday, 12:00 **N-3**

Integrating host plant resistance with biocontrol: silicon supplementation and entomopathogenic nematodes to control major turfgrass insects

Tarikul Islam, Matthew Brown, ***Albrecht Koppenhöfer***

Lunch

Tuesday, 13:30 – 14:30
Foyer

Excursions Tuesday, 14:30 – 17:30
Botteled water will be provided before starting

Excursion 1
Schloss Schönbrunn
Guides will pick you up at the conference site. Public transportation (7-day-ticket) will be used. Meet at 14:25 for grouping of attendees.

Excursion 2
City Walk
Guides will pick you up at the conference site. Public transportation (7-day-ticket) will be used. Meet at 14:25 for grouping of attendees. At the end of the tour, the guides will bring you to the BBQ place via public transportation.

Excursion 3
Vienna Zoo
Guides will pick you up at the conference site. Public transportation (7-day-ticket) will be used. Meet at 14:25 for grouping of attendees.

Tuesday, 18:30 – 22:00
BBQ – Brandauer Schlossbräu, Am Platz 5, 1130 Vienna
It is in walking distance from Schloss Schönbrunn and the Vienna Zoo.
No transportation will be provided to and from the BBQ place as it can be easily reached using public transportation.

Wednesday, July 31, 2024

Registration Desk

Wednesday, 8:00 – 18:00
Foyer

Cross-Div. Symposium Microbial Control and Nematodes

Wednesday, 8:30 – 10:00
Plötzl EI 8

New under the Sun - Progress in Mass Production and Formulation of Microbials
Chairs: Dana Ment, Patricia Navarro, Chad Keyser

CDS Microbial Control and Nematodes. Wednesday, 8:30 **17-1**

Worldwide microbial pest control agents or environmentally competent ones?

Enrique Quesada-Moraga

CDS Microbial Control and Nematodes. Wednesday, 9:00 **17-2**

From Legacy to Weapon: Unveiling a New Biopesticide

Omri Mayer

CDS Microbial Control and Nematodes. Wednesday, 9:30 **17-3**

Innovative mass production and novel formulation of baculovirus biopesticides for false codling moth management

***Sean Moore**, John Opoku-Debrah, Tamryn Marsberg, Michael Jukes, Marcel van der Merwe, Theunis Lombard, Mathew Goddard, Anne Grobler, Wanda Booyens, Caroline Knox, Martin Hill*

Contributed Papers Viruses 3

Wednesday, 8:30 – 10:15
Hlawka El 9

Viral covert Infections
Chairs: Wang Xi, Michael Jukes

CONTRIBUTED PAPERS. Wednesday, 8:30 **V-16-STU**

The perks of being a wallflower: exploration of "silent" Iflavirus infections in *S. exigua*

***Annamaria Mattia**, Astrid Bryon, Mitchel Schreurs, Jemma Fourie, Salvador Herrero, Vera I.D. Ros*

CONTRIBUTED PAPERS. Wednesday, 8:45 **V-17**

Unveiling Covert Baculovirus Infection: Insights from Laboratory Trials on *Helicoverpa armigera* under Stress Conditions

***Olivia Osterwalder**, Anna Landwehr, Silvan Bosshard, Heiri Wandeler*

CONTRIBUTED PAPERS. Wednesday, 9:00 **V-18-STU**

MARTIGNONI AWARD

Effect of domestication on the repertoire of viruses and bacteria associated with the Mediterranean fruit fly, *Ceratitis capitata*

***Luis Hernández-Pelegrín**, Fang-Shiang Lim, Pablo García-Castillo, Joel González-Cabrera, Vera I.D. Ros, Jörg T. Wennmann, Salvador Herrero*

CONTRIBUTED PAPERS. Wednesday, 9:15 **V-19**

Potential of a new *Glossina morsitans morsitans* cell line for isolation and propagation of tsetse fly viruses

***Lesley Bell-Sakyi**, Giovanni Petrucci, Catherine Hartley, Benjamin Makepeace, Alistair C. Darby, Adly Abd-Alla*

CONTRIBUTED PAPERS. Wednesday, 9:30 **V-20**

Isolation of Iflaviruses of the testse fly *Glossina morsitans morsitans*

***Hannah-Isadora Huditz**, Giovanni Petrucci, Davor Skaric, Ben Raymond, Monique M. van Oers, Adly M.M. Abd-Alla*

CONTRIBUTED PAPERS. Wednesday, 9:45 **V-21 STU**

Novel RNA viruses of the tomato leafminer *Tuta absoluta*

***Rosa Esmeralda Becerra García,** Luis Hernández Pelegrín, Cristina Crava, Salvador Herrero*

CONTRIBUTED PAPERS. Wednesday, 10:00 **V-22 STU**

Re-emergence of homeostatic microbiome of mud crab *Scylla serrata* post-dysbiotic shift triggered by White Spot Syndrome Virus (WSSV) infection

***Angela Camille Aguila-Toral**, Gardel Xyza Libunao, Edgar Amar, Rachel Ravago-Gotanco*

Contributed Papers Bacteria 3

Wednesday, 8:30 – 10:30
Fritz Paschke El 10

Engineering and applied Aspects
Chairs: Luca Ruiu, Dafne Toledo

CONTRIBUTED PAPERS. Wednesday, 8:30 **B-17**

Bacillus thuringiensis serovar _kurstaki_ (Btk) Strains for Industrial Production in wheat bran based medium: Insights from Genomic Exploration and Nutritional Optimization

*Rita Barssoum, Nancy Fayad, Rayan Nasreddine, Séphanie Dupoiron, César Arturo Aceves-Lara, Julien Cescut, Luc Fillaudeau, Dietrich Stephan, **Mireille Kallassy***

CONTRIBUTED PAPERS. Wednesday, 8:45 **B-18**

***Bacillus thuringiensis* Spores and Cry Toxins Act Synergistically to Expedite Colorado Potato Beetle Mortality: Mechanisms, Application and Perspectives**

***Ivan Dubovskiy**, Ekaterina Grizanova, Daria Tereshchenko, Tatiana Krytsyna*

CONTRIBUTED PAPERS. Wednesday,9:00 **B-19**

The use of *Bacillus thuringiensis* to control root-knot nematodes

*Yolanda Bel, Magda Galeano, Mireya Baños-Salmeron, **Baltasar Escriche***

CONTRIBUTED PAPERS. Wednesday, 9:15 **B-20**

Bacillus thuringiensis Cry proteins - an arsenal of anti-parasitics

***Raffi Aroian**, Kelly Flanagan, Qian Ding, Duy Hoang, Nicholas Cazeault, Hanchen Li, Stefani Diaz Valerio, Florentina Rus, Ernesto Soto, Yasushi Kageyama, Heiko Liesegang, Gary Ostroff*

CONTRIBUTED PAPERS. Wednesday, 9:30 **B-21**

Building an insecticidal protein gene library: deep expansion

*Yang Geng, Jialu Li, Hanxiao Wang, Jialin Pu, Jinshui Zheng, Donghai Peng, **Ming Sun**[2]*

CONTRIBUTED PAPERS. Wednesday, 9:45 **B-22**

Selecting a customised Crybody for the Mediterranean fruit fly *Ceratitis capitata*

*Carolina Macarena Araya-Aracena, **Susana Vilchez***

CONTRIBUTED PAPERS. Wednesday, 10:00 **B-23**

How pest resistance to *Bacillus thuringiensis* helps to improve bioinsecticides?

***Ekaterina Grizanova**, Tatiana Krytsyna, Ivan Dubovskiy*

CONTRIBUTED PAPERS. Wednesday, 10:15 **B-24**

Risk Assesment of New Btk Isolates BLB1 and LIP Biopesticides via Toxicity Assays on Lab Animals

***Gül Ayyildiz**, Hazar Kraïem, Sayda Dhaouadi, Rim El Jeni, Zeynep Yurtkuran-Çeterez, Dietrich Stephan, Zakaria Benlasfar, Balkiss Bouhaouala-Zahar*

Refreshment Break

Wednesday, 10:30 – 11:00
Foyer

Contributed Papers Microbial Control 3

Wednesday, 11:00 – 13:00
Plötzl EI 8

Integrated Pest Management and emerging Biocontrol Technologies
Chair: Jarrod Leland

CONTRIBUTED PAPERS. Wednesday, 11:00 **MC-15**

Constitutive and inducible tomato defenses contribute to *Bacillus thuringiensis* lethality against *Spodoptera exigua*

***Ada Frattini Llorens**, Rosa María González Martínez, Juan García, Zhivko Minchev, María José Pozo, Víctor Flors, Cristina Crava, Salvador Herrero*

CONTRIBUTED PAPERS. Wednesday, 11:15 **MC-16**

Benefits of Baculovirus Use in IPM Strategies

*Anna Landwehr, **Heiri Wandeler***

CONTRIBUTED PAPERS. Wednesday, 11:30 **MC-17**

CpGV Resistance – Where we are and where to go

***Johannes A. Jehle**, Sarah Biganski, Birgit Ruoff, Jutta Kienzle, Jiangbin Fan, Jörg T. Wennmann, Karin Undorf-Spahn, Eva Fritsch*

CONTRIBUTED PAPERS. Wednesday, 11:45 **MC18**

Chromosome-level comparison between susceptible and resistant codling moth strains to decoding the mechanism of CpGV resistance of codling moth

***Jiangbin Fan**, Fang-Shiang Lim, Jörg T. Wennmann, David Heckel, Petr Nguyen, Johannes A. Jehle*

CONTRIBUTED PAPERS. Wednesday, 12:00 **MC-19**

Fungal Entomopathogens for pest biocontrol in multitrophic approaches

***Frederic Francis**, Marcellin C. Cokola, Kenza Dessauvages, Kouanda Nongamanégré, Lallie Glacet, Athanase Badolo, Ibtissem Ben Fekih*

CONTRIBUTED PAPERS. Wednesday, 12:15 **MC-20**

Prospects of NoVil (*Metarhizium robertsii* strain CPD006) in controlling *Frankliniella occidentalis, Myzus persicae* and *Tetranychus urticae* on greenhouse crops

***Jean Nguya K. Maniania**, Fayaz M. Amnulla, Andrei Darie, Nicole Stewart, Jeff Reitsma, Ishtiaq M. Rao*

CONTRIBUTED PAPERS. Wednesday, 12:30 **MC-21**

Api-vectoring of *Beauveria bassiana* for thrips control in strawberry tunnels

*Morgane Ourry, Marta Montoro, Katja K. Nielsen, Zhicong Wu, Antoine Lecocq, Annette B. Jensen, **Nicolai V. Meyling***

CONTRIBUTED PAPERS. Wednesday, 12:45 **MC-22**

Antagonistic potential of entomopathogenic and endophytic fungi against fusarium wilt pathogen of tomato *Fusarium oxysporum f. sp. lycopersici*

*Marie Cecile Muhorakeye, Everlyne Samita Namikoye, Fathiya Mbarak Khamis, Waceke Wanjohi, **Komivi Senyo Akutse***

DBI Division Symposium

Wednesday, 11:00 – 13:00
Hlawka EI 9

Sex-distorting Parasites: Pathological and ecological Consequences
Chair: Jamie Bojko

DBI DIVISION SYMPOSIUM. Wednesday, 11:00 **11-1**

Sex ratio distorters and the evolution of host sex determination mechanisms
Richard Cordaux

DBI DIVISION SYMPOSIUM. Wednesday, 11:30 **11-2**

A tale of two endosymbionts that affect host fitness and sex allocation via egg-size provisioning in a haplodiploid insect species
*Alihan Katlav, Amir Tourani, James Cook, **Markus Riegler***

DBI DIVISION SYMPOSIUM. Wednesday, 12:00 **11-3**

Invasion of the body snatchers: the role of parasite introduction in host distribution and response to salinity in invaded estuaries
April Blakeslee

DBI DIVISION SYMPOSIUM. Wednesday, 12:30 **11-4**

Pathological dynamics of the sexually transmitted betanudivirus Heliothis zea nudivirus 1
***Jirka Manuel Petersen**, Annie Bézier, Jean-Michel Drezen, Astrid Bryon, Monique van Oers*

Fungi Division Symposium

Wednesday, 11:00 – 13:00
Fritz Paschke EI 10

Fungal Interaction and Management of beneficial Insects
Chairs: Nemat Keyhani, Jae Su Kim

FUNGI DIVISION SYMPOSIUM. Wednesday, 11:00 **13-1**

Integrative investigations into the strategies of zombie-making *Ophiocordyceps* to manipulate carpenter ant behavior

Charissa de Bekker

FUNGI DIVISION SYMPOSIUM. Wednesday, 11:24 **13-2**

A biosynthetic survey of entomopathogenic fungi

Pablo Cruz-Morales

FUNGI DIVISION SYMPOSIUM. Wednesday, 11:48 **13-3**

Entomopathogenic fungi and insect predators teaming up against aphids: Friend or foe?

***Ibtissem Ben Fekih**, Annette B. Jensen, Jørgen Eilenberg, Kris A.G. Wyckhuys, Frederic Francis, Gabor Pozsgai*

FUNGI DIVISION SYMPOSIUM. Wednesday, 12:12 **13-4**

Isolation of a probiotic bacterium to protect silkworms against fungal parasites

*Pengfei Zhao, Song Hong, **Chengshu Wang***

FUNGI DIVISION SYMPOSIUM. Wednesday, 12:36 **13-5**

Interaction of endophytic *Beauveria bassiana* with predators and parasitoids for hemipteran pest control in horticulture

***Natalia González-Mas**, María Cuenca-Medina, Enrique Quesada-Moraga*

Lunch

Wednesday, 13:00 – 14:00
Foyer

JIP Editorial Board

Wednesday, 13:00 – 14:00
Seminar Room 363

Student Workshop

Wednesday, 13:00 – 14:00
Plötzl EI 8

Contributed Papers Microsporidia

Wednesday, 14:15 – 15:45
Plötzl EI 8

Recent Advances in Microsporidia Research
Chairs: Jonathan Snow, Courtney MacInnis

CONTRIBUTED PAPERS. Wednesday, 14:15 **MS-1-STU**

Data mining reveals diversity and host spectrum of cryptic microsporidian parasites across the Panarthropods

***Sam Edwards**, Edouard Bessette, Bryony Williams*

CONTRIBUTED PAPERS. Wednesday, 14:30 **MS-2**

Lithium Chloride reduces *Vairimorpha (Nosema) ceranae* infection in honey bees

*Parker Parrella, Victoria Cordero, **Jonathan Snow***

CONTRIBUTED PAPERS. Wednesday, 14:45 **MS-3**

A protease associated with microsporidian spore germination

*Fangyan Liu, **Jie Chen**, Quan Sun, Rong Wang, Bing Han, Ying Wang, Xiaoqun Dang, Xianzhi Meng, Guoqing Pan, Zeyang Zhou*

CONTRIBUTED PAPERS. Wednesday, 15:00 **MS-4-STU**

The diversity and co-infections of microsporidia in coexisting cryptic lineages of an amphipod crustacean: what can we learn from Nanopore amplicon sequencing?

***Nataša Katanić**, Pavel Karel Bystřický, Denis Copilaş-Ciocianu, Magdalena Gajdošová, Jacqueline Grimm, Christoph Hahn, Tereza Rutová, Kristina Sefc, Adam Petrusek*

CONTRIBUTED PAPERS. Wednesday, 15:15 **MS-5**

Molecular, Morphological identification and whole genomic sequencing of a microsporidium from cucumber moth, *Diaphania indica* (Saunders)

***Yu-Yun Kuo**, Chun-Yan Lee, Yu-Shin Nai*

CONTRIBUTED PAPERS. Wednesday, 15:30 **MS-6**

First record of *Nosema maddoxi* (Microsporidia, Nosematidae) in populations of *Nezera viridul*a (L) and *Palomena prasina* (L) in Georgia

***Manana Kereselidze**, Marek Barta, Nika Guntadze, Daniela Pilarska, Andreas Linde, Ann Hajek*

Contributed Papers Viruses 4

Wednesday, 14:00 – 16:00
Hlawka EI 9

Virus - Host Interaction
Chairs: Jean-Michel Drezen, Bryony Bonning

CONTRIBUTED PAPERS. Wednesday, 14:00 **V-23-STU**

Comparative transcriptome analysis unveils varied host responses to sacbrood virus infection in *Apis cerana* and *Apis mellifera*

***Zih Ting Chang**, Yu-Feng Huang, Tzu-Han Chen, Li-Hung Chang, Chung-Yu Ko, Yu-Shin Nai, Yue-Wen Chen*

CONTRIBUTED PAPERS. Wednesday, 14:15 **V-24-STU**

Exploring the virome of honey bee *(Apis mellifera*) and *Varroa* mite (*Varroa destructor*) in Taiwan through viral metagenomics

***Fang-Min Chang**, Yen-Hou Chen, Ming-Cheng Wu, Yu-Shin Nai*

CONTRIBUTED PAPERS. Wednesday, 14:30 **V-25**

Comparison of bracovirus in incipient parasitoid species specialized on different hosts

*Camille Heisserer, Elisabeth Huguet, Karen Kester, Dawn Gundersen-Rindal, Thibaut Josse, **Jean-Michel Drezen***

CONTRIBUTED PAPERS. Wednesday, 14:45 **V-26-STU**

Transcriptomic evidence for a conserved, nudiviral RNA polymerase in the parasitoid wasp, *Microplitis demolitor*, and the implications on *Bracovirus* gene discovery

***Kelly Tims**, Gaelen R. Burke*

CONTRIBUTED PAPERS. Wednesday, 15:00 **V-27**

Suppressons: Ancient Nudivirus Virions Exapted to Form Parasitoid Organelles that Suppress Host Immunity

Brian Federici

CONTRIBUTED PAPERS. Wednesday, 15:15 **V-28**

Using AmE-711 Honey Bee Cells to Examine Virus - Fungicide Interactions at the Cell Level

***Michael Goblirsch**, John Adamczyk*

CONTRIBUTED PAPERS. Wednesday, 15:30 **V-29**

Inhibition of medically important mosquito-borne viruses by the Insect-Specific Flavivirus Binjari across acute and persistent states in mosquito cells

*Wessel Willemsen, Nick Helmes, Marleen Henkens, Ruben Spruijt, Monique M. van Oers, Gorben Pijlman, **Jelke Fros****

CONTRIBUTED PAPERS. Wednesday, 15:45 **V-30**

***Aedes albopictus* response to Cell fusing Agent virus infection in relation to temperature**

***Hugo Perdomo**, Ayda Khorramnejad, Nfamara Cham, Alida Kropf, Davide Sogliani, Mariangela Bonizzoni*

Bacteria Division Symposium

Wednesday, 14:00 – 16:00
Fritz Paschke EI 10

Physiology, Safety and Taxonomy of bacterial Pesticides
Chairs: Ben Raymond, William Moar

BACTERIA DIVISION SYMPOSIUM. Wednesday, 14:00 **14-1**

***Bacillus thuringiensis* at the cross roads: Insights into enteropathogenicity of *Bacillus cereus* group**

Monika Ehling-Schulz

BACTERIA DIVISION SYMPOSIUM. Wednesday, 14:30 **14-2**

Phenotypic heterogeneity and sporulation-independent persistence in *Bacillus thuringiensis* during infection

*Hasna Toukabri, Didier Lereclus, **Leyla Slamti***

BACTERIA DIVISION SYMPOSIUM. Wednesday, 15:00 **14-3**

Assessing insecticidal protein safety without HOSU or source organism

***Concepción Novillo**, Pascale Delzenne, Katherine Karberg, Huixin Lin, William Moar, Yong Yin*

BACTERIA DIVISION SYMPOSIUM. Wednesday, 15:30 **14-4**

Towards a consensus taxonomy of the *Bacillus cereus* group of bacteria in the sequencing era

***Annika Gillis**, Niels Bohse Hendriksen, Ole Andreas Økstad*

Refreshment Break

Wednesday, 16:00 – 16:30
Foyer

Poster Session

Wednesday, 16:30 – 18:00
Foyer

POSTER SESSION **B-P1**

Receptor recognition site of insecticidal pore-forming toxin Cry46Ab

***So Takebe**, Yoshinao Azuma, Tohru Hayakawa*

POSTER SESSION **B-P2-STU**

The Construction of Secretory Expression Engineering Bacteria for the Trans-Cry3Aa-T-HasA Fusion Protein against the *Monochamus alternatus*

Xiaohong Han

POSTER SESSION **B-P3-STU**

Potential of feed-born bacteria for phytopathogen and insect pest management

***Manuela Casada**, Francesco Fancello, Lucia Maddau, Severino Zara, Luca Ruiu*

POSTER SESSION **B-P4-STU**

Bacterial entomopathogens for the management of insecticide-resistant mosquito populations

***Alessia Vinci**, Cipriano Foxi, Valentina Sini, Giuseppe Satta, Luca Ruiu*

POSTER SESSION **B-P5**

Nanoparticle-loaded microcapsules providing effective UV protection for Cry protein

*Yongjing Zhang, Aijing Zhang, Mengyuan Li, Kanglai He, **Shuyuan Guo***

POSTER SESSION **B-P6**

NupR is involved in the control of PlcR, a pleiotropic regulator of extracellular virulence factors

*Jiaxin Qin, Yizhuo Zhang, Cheng Qian, Bing Yan, **Jun Cai***

POSTER SESSION **DBI-P1-STU**

Flagella of *Paenibacillus larvae* – influence on multicellular behavior and virulence

***Josefine Göbel**, Anne Fünfhaus, Julia Ebeling, Elke Genersch*

POSTER SESSION **DBI-P2**

Epidemiological study of *Paenibacillus larvae* via MLVA using the example of a major European city

***Anne Fünfhaus**, Julia Ebeling, Elke Genersch*

POSTER SESSION **DBI-P3-STU**

Establishment of an exposure bioassay for experimental infection of honey bee larvae with SBV

*Sarah Riebschläger, **Runlin Li**, Sebastian Gisder, Elke Genersch*

POSTER SESSION **DBI-P4-STU**

Evolution of virulence in *Paenibacillus larvae*, a honey bee pathogenic bacterium

***Antonia Reinecke**, Juliane Schreiber, Tristan Aretz, Elke Genersch*

POSTER SESSION **DBI-P5-STU**

The necrotrophic lifestyle of *Paenibacillus* larvae: Suppression of carcass microbiota in honey bee larvae

***Niklas Sibum**, Anne Fünfhaus, Elke Genersch*

POSTER SESSION **F-P1**

Evaluation of the toxicity and mode of action of novel fungal lectins and protease inhibitors against *Drosophila suzukii* (Diptera: Drosophilidae)

*Jaka Razinger, Igor Nekrep, **Eva Praporotnik**, Nada Žnidaršič, Urban Bogataj, Polona Mrak, Tjaša Šentjurc, Sergej Praček, Katarina Karničar, Dušan Turk, Jerica Sabotič*

POSTER SESSION **F-P2**

Natural incidence of *Beauveria bassiana* as biological control agent of tarnished plan bug and stink bugs in Mississippi Delta

***Maribel Portilla**, Yuzhe Du, James Glover, Gadi V. P. Reddy*

POSTER SESSION **F-P3**

Transcriptome analysis of *Beauveria bassiana* infected coffee berry borer *Hypothenemus hampei* (Ferrari)

***Fang-Min Chang**, Hsiao-Ling Lu, Yu-Shin Nai*

POSTER SESSION **F-P4**

Characterization of *Beauveria bassiana* 331R to control the two-spotted spider mite, *Tetranychus urticae*

***Seo Yeong Mun**, Kyu Seek Kim, Ra Mi Woo, Hyuk Jin Moon, Soo Dong Woo*

POSTER SESSION **F-P5**

Storage stability of encapsulated *Batkoa* sp. spores dried in a fluidized bed

***Daniela Milanez Silva**, Linda Muskat, Natasha Sant Anna Iwanicki, Anant Patel, Italo Delalibera Júnior*

POSTER SESSION **F-P6**

***Beauveria caledonica:* Microsclerotia formation and virulence against two lepidopteran species**

*Lorena García-Riaño, Juliana Gómez-Valderrama, Carlos Espinel, **Gloria Barrera-Cubillos**, Laura Villamizar*

POSTER SESSION **F-P7-STU**

Fungal formulations against *Drosophila suzukii*, related drosophilids, without detrimental effects on *Apis mellifera*

***Ricardo Alberto Toledo Hernández**, Rodrigo Lasa, Pablo Montoya, Pablo Liedo, Daniel Sánchez, Douglas Rodríguez, Mónica Pulido, Jorge Toledo*

POSTER SESSION **F-P8-STU**

Medium optimization and bioefficacy of *Hirsutella thompsonii* microsclerotia and blastospores for biological control of mites and ticks

***Daniela Milanez Silva**, Yuri Dantas, Natasha Sant Anna Iwanicki, Italo Delalibera Júnior*

POSTER SESSION **F-P9**

The MICOTI project: towards a fungal-based integrated pest management against *Scaphoideus titanus.*

*Marika Rossi, Marta Vallino, Paola Dolci, **Simona Abbà***

POSTER SESSION **MC-P1-STU**

In search of entomopathogens of the oak processionary moth in Germany

***Shin Yee Tan**, Regina G. Kleespies, Aikaterini Dounavi, Nicola M. Fischer, Jérôme Morinière, Johannes A. Jehle, Jörg T. Wennmann, Dietrich Stephan*

POSTER SESSION **MC-P2**

Investigations on the efficiency of microbial control agents, natural substances and copper compounds for the control of *Halyomorpha halys*

***Martin Parth**, Angelika Gruber, Manfred Wolf*

POSTER SESSION **MC-P3**

Novel biopesticide solutions for UK pests and diseases, introducing two UKRI projects

***Steve Edgington**, Rhian Whelan, Archita Barua*

POSTER SESSION **MC-P4**

Bioprospecting environmental fungi of Crete for the development of mosquito biopesticides

***Joel Couceiro**, Iliana Sidira, Martyn Wood, Juan Silva, Andronikos Papadopoulos, Stefanos Mastis, John Vontas, George Dimopoulos*

POSTER SESSION **MC-P5**

Using nanoparticles to enhance dsRNA uptake efficiency in *Phthorimaea absoluta*

***Ayaovi Agbessenou**, Jiangbin Fan, Christian Borgemeister, Johannes A. Jehle*

POSTER SESSION **MC-P6**

Environmental Constraints to *Beauveria* Efficacy for *Cimex lectularis* (Bedbug)

***Stefan Jaronski**, Morgan Wilson, Dini Miller*

POSTER SESSION **MC-P7**

Spore Persistence of *Beauveria* Wettable Powder and Emulsifiable Concentrate Formulations Under Lower Rio Grande Valley, Texas, Climate Variables

*Yareny Ramirez, Daniela Sanchez, **Stefan Jaronski**, Mayra Reyes, Isaiah Garza, Justin Wendel, Christopher Vitek, Daniel Flores*

POSTER SESSION **MC-P8**

Engineering of multiple trypsin/chymotrypsin sites in Cry3A to enhance its activity against *Monochamus alternatus* Hope larvae
Songqing Wu

POSTER SESSION **MC-P9**

Cadherin gene is involved in the toxicity of *Bacillus thuringiensis* subsp. *aizawai* to *Spodoptera frugiperda*
Youngjin Park

POSTER SESSION **MC-P10-STU**

Insights into Insects-Fungus Interplay: Transcriptomic Profiling of *Metarhizium anisopliae* Treatment
***Hyeon Wook Jung**, Hoe Ri Kim, Se Jin Lee*

POSTER SESSION **MC-P11**

Proteomics for studying the microbiome in non-model insects
***Simona Abbà**, Marta Vallino, Simona Cirrincione, Cristina Lamberti, Marika Rossi*

POSTER SESSION **MC-P12**

Effect of beneficial microbes on aphid development and predatory hoverflies host choice-selection
***Ibtissem Ben Fekih**, Marc Ongena, Magali Deleu, Marie-Laure Fauconnier, Kenza Dessauvages, Marcellin C. Cokola, Frederic Francis*

POSTER SESSION **MS-P1**

Ultrastructure of *Tubulinosema loxostegi* Malysh Tokarev Issi 2013 re-isolated from West Siberian populations of beet webworms and propagated in Siberian silkmoth *Dendrolimus sibiricus*
***Yuliya Sokolova**, Arina Rumiantseva, Yuliya Malysh, Yuri Tokarev*

POSTER SESSION **MS-P2**

Olfactory recognition of microsporidia-infected *Drosophila suzukii* by parasitic wasps has consequences for wasps´ fitness
***Sarah Biganski**, Lara Alicia Winterwerber, Jannicke Gallinger, Jürgen Gross, Johannes A. Jehle*

POSTER SESSION **MS-P3-STU**

Effect of the chitosan-based dsRNA nanocomplex on microsporidian parasites *Nosema ceranae* in the honey bee
***Hyun Goo Kim**, Kyu Seek Kim, Ra Mi Woo, Hyuk Jin Moon, Soo Dong Woo*

POSTER SESSION **MS-P4**

Analysis of the genetic diversity of the honey bee (*Apis mellifera*) pathogen *Nosema* spp. based on Short Tandem Repeats (STR)
***Sebastian Gisder**, Lucas Lannutti, Leonhard Schnittger, Elke Genersch*

POSTER SESSION **MS-P5**

Genomic variations among *Vairimorpha ceranae* different isolates revealing microsporidia might adapt to thermal stress in Taiwan
***Yi-Hsuan Li**, Fang-Min Chang, Ming-Cheng Wu, Yu-Shin Nai*

POSTER SESSION **N-P1**

Resolution of efficiency of entomopathogenic nematode *Steinernema germanica* sp. (Rhabditida: Steinernematidae) on the potato tuber moth (*Phthorimaea operculella* (Zeller)) (Lepidoptera: Gelechiidae) under laboratory conditions
***Nona V Mikaia**, Irina A. Khelisupali, Zaira R. Tkhebuchava, Narimanishvili V. Tamara*

POSTER SESSION **V-P1**

Genomic analysis of different American strains of SfNPV baculovirus isolated from *Spodoptera frugiperda*
*Ma. de los Angeles Bivián-Hernández, Jonatan C. Rangel-Núñez, Elisabeth Herniou, **Ma.Cristina Del Rincón-Castro***

POSTER SESSION **V-P2**

Identification of the molecular basis of CpGV resistance in codling moth, *Cydia pomonella*
***Jiangbin Fan**, Jörg T. Wennmann, Johannes A. Jehle*

POSTER SESSION **V-P3**

The genomes of an alphabaculovirus and a betabaculovirus from the tufted apple bud moth, *Platynota idaeusalis*, reveal recent recombination between baculoviruses that share a host
***Robert L. Harrison**, Daniel Rowley*

POSTER SESSION **V-P4**

Autographa californica Multiple Nucleopolyhedrovirus *ac106* Is Required for Intranuclear Microvesicle Formation, and Intranuclear Microvesicle Formation is Essential for the Intranuclear Transport of ODV Integral Envelope Proteins
*Jiannan Chen, Mei Mo, Yushan Yang, Yinyin Yu, Wenbi Wu, Kai Yang, **Meijin Yuan***

POSTER SESSION **V-P5**

Recombinant SpfrGV enhancin proteins improve the insecticidal activity of SfMNPV-ColA on *Spodoptera frugiperda* larvae
*Kewin Rodriguez-Obediente, Mariano Nicolás Belaich, Juliana Gómez-Valderrama, **Gloria Barrera-Cubillos***

POSTER SESSION **V-P6-STU**

Increasing production efficiency of target proteins by regulating VLF-1 in baculovirus expression system
***Seo Yeong Mun**, Min Kong, Hyuk Jin Moon, Soo Dong Woo*

POSTER SESSION **V-P7**

Function of AcMNPV-miR-2 in AcMNPV infection
*Xinghua Yu, Tingkai Teng, Zhuowen Duan, **Jinwen Wang***

POSTER SESSION **V-P8**

Combining SIT and baculovirus application in an integrated approach to management of the false codling moth
***Windy Sekgele**, Sean Moore, Tamryn Marsberg*

POSTER SESSION **V-P9-STU**

Whole genomic sequencing and analysis of Rhagastis binoculata nucleopolyhedrovirus (NPV) in Taiwan
***Yu-Yun Kuo**, Ju-Chun Chang, Yi-Hsuan Li, Yu-Feng Huang, Tzong-Yuan Wu, Yu-Shin Nai*

POSTER SESSION **V-P10-STU**

Improving recombination efficiency in the baculovirus expression system for foreign gene expression
***Juul Steeghs**, Linda King, Robert Possee, Adam Chambers, Gorben Pijlman*

POSTER SESSION **V-P11-STU**

Transmission of nudivirus among *Oryctes rhinoceros* at the different developmental stages
***Mayuho Yamauchi**, Christopher Kitalong, Madoka Nakai*

POSTER SESSION **V-P12-STU**

Quality control of baculovirus propagation based on single nucleotide variants (SNVs)
***Christian Oehlmann**, Birgit Ruoff, Jörg T. Wennmann, Johannes A. Jehle*

POSTER SESSION **V-P13**

Development of a multiplex PCR assay for the detection of nudiviruses in *Drosophila suzukii* using artificial positive controls
***Sevgi Gezer**, Sarah Biganski, Johannes A. Jehle, Annette Herz, Jörg T. Wennmann*

POSTER SESSION **V-P14**

Covert infections caused by cypovirus in Noctuidae and Erebidae soybean pests
*Alini de Almeida, Andrews Fisch, Maria Cristina Neves de Oliveira, Bergmann Morais Ribeiro, **Daniel Ricardo Sosa-Gómez***

POSTER SESSION **V-P15**

Identification of mycoviruses through the transcriptomic data of entomopathogenic fungi, *Beauveria bassiana* NCHU-271 and *Metarhizium pinghaense* NCHU-125
***Yu-Shin Nai**, Fang-Min Chang, Cheng-Yu Hsieh*

POSTER SESSION **V-P16**

The importance of ORF amv*133* on Amsacta moorei entomopoxvirus replication
*Emine Ozsahin, **Peter Krell**, Eva Nagy, Zihni Demirbağ*

POSTER SESSION **V-P17**

Developing potential intervention strategies for SFTSV group bandaviruses based on their phylogeny

Xiaoli Wu, Liyan Fu, Jin Qian, Shuang Tang, Shu Shen, ***Fei Deng***

POSTER SESSION **V-P18-STU**

Expression of respiratory syncytial virus fusion protein using an insect virus surface-display system

Hyuk Jin Moon*, Soo Dong Woo*

POSTER SESSION **V-P19**

Viromes of tick tissues revealed different capabilities to vector and transmit viruses in nine tick species.

Jun Ni, Hongfeng Chen, Shouwei Huang, Yaohui Fang, Fei Deng, ***Shu Shen***

POSTER SESSION **V-P20-STU**

Estimation of regions responsible for the lethal activity of parasitoid killing factors encoded in entomopoxvirus by point mutation

Haru Migita*, Taku Onodera, Madoka Nakai*

POSTER SESSION **V-P21-STU**

Development of a DWV-specific immunohistochemistry protocol to analyze tissue and cell tropism of DWV

Sarah Riebschläger*, Sebastian Gisder, Elke Genersch*

POSTER SESSION **V-P22**

Use of Insect Gut Binding Peptides to Identify Pathogen Binding Domains and Receptors

Bryony Bonning*, Ruchir Mishra*

POSTER SESSION **V-P23-STU**

Efficient production of hand, foot, and mouth disease virus-like particles (HFMD-VLPs) in insect cells

Hyun Jung Kim*, Hyuk Jin Moon, Soo Dong Woo*

POSTER SESSION **V-P24-STU**

Development of a Bioinformatic Pipeline for Defining Orthology Groups in *Baculoviridae* Protein Genes.

Franco Uriel Cuccovia Warlet*, Lucas Federico Motta, Jorge Alejandro Simonin, Fernando Maku Lassalle, Carolina Susana Cerrudo, Mariano Nicolás Belaich*

ICTV Study Groups Meeting
Wednesday, 19:30 – 21:00
Seminar Room 363

Fungi Division Business Meeting
Wednesday, 19:30 – 21:00
Plötzl EI8

Microbial Control Division Business Meeting
Wednesday, 19:30 – 21:00
Hlawka EI 9

DBI Division Business Meeting
Wednesday, 19:30 – 21:00
Fritz Paschke EI 10

Thursday, August 1, 2024

Registration Desk
Thursday, 8:00 – 14:00
Foyer

Contributed Papers Viruses 5
Thursday, 9:15 – 10:30
Lecture Hall EI 7

Pathogenicity, Virulence and Biological Control
Chairs: Elisabeth Huguet, Umut Toprak

CONTRIBUTED PAPERS. Thursday, 9:15 **V-31**

Efficacy of sixteen nucleopolyhedrovirus isolates against *Helicoverpa armigera, Spodoptera litura*, and *Trichoplusia ni* in Sri Lanka

W.M.N.Kaumadie K. Kulasinghe, K. M. D. W. Prabath Nishantha, Robert L. Harrison, K. S. Hemachandra, Lionel Nugaliyadde, ***Omaththage P. Perera***

CONTRIBUTED PAPERS. Thursday, 9:30 **V-32**

The expression of CrpeNPV gp37 as a formulation additive for improved infectivity with CrleGV against *Thaumatotibia leucotreta*

Naho Muleya, Siyabonga Phoswa, Caroline Knox, Sean Moore, Martin Hill, ***Michael Jukes***

CONTRIBUTED PAPERS. Thursday, 9:45 **V-33**

Carbon quantum dot nanoparticles increase the efficacy of Spodoptera littoralis nucleopolyhedrovirus suspoemulsion

Ali Mehrvar, ***Umut Toprak****, Gökhan Söylemezoğlu, Solmaz Ghanbari*

CONTRIBUTED PAPERS. Thursday, 10:00 **V-34**

A CRM1-Dependent Nuclear Export Signal in Autographa californica Multiple Nucleopolyhedrovirus Ac93 Is Important for the Formation of Intranuclear Microvesicles

Guoqing Chen*, Jing Yang, Yihong Wu, Haoran Wang, Xinxin Zhang, Guozhong Feng*

CONTRIBUTED PAPERS. Thursday, 10:15 **V-35**

Expression of viral antiapoptotic genes during the development of SfNPV-Ar baculovirus infection in its host *Spodoptera frugiperda*

Jonatan C. Rangel-Núñez, Ma. de los Angeles Bivián-Hernández, Jorge E. Ibarra, ***Ma. Cristina Del Rincón-Castro***

Contributed Papers DBI 2
Thursday, 8:30 – 10:15
Plötzl EI 8

Pathogen and Symbiont: Host Interactions in Invertebrate Mass Production
Chairs: Elisabeth Herniou, Christina Nielsen-LeRoux

CONTRIBUTED PAPERS. Thursday, 8:30 **DBI-9-STU**

***In vitro* cultivation of tsetse fly *Glossina fuscipes fuscipes* endosymbiont *Spiroplasma:* Genome sequencing and potential implications for disease transmission**

Fabian Gstöttenmayer

CONTRIBUTED PAPERS. Thursday, 8:45 **DBI-10-STU**

Iflavirus and Negevirus dynamics in mass-reared tsetse flies

Hannah-Isadora Huditz*, Giovanni Petrucci, Adly M.M. Abd-Alla, Ben Raymond, Monique M. van Oers*

CONTRIBUTED PAPERS. Thursday, 9:00 **DBI-11-STU**

Reinfection and genetic diversity of Tenebrio molitor densovirus (TmDV) from Europe

***Fang-Shiang Lim**, Joel González Cabrera, Johannes A. Jehle, Jörg T. Wennmann*

CONTRIBUTED PAPERS. Thursday, 9:15 **DBI-12**

Applying Ecotoxicology Principles in Mass-Reared Insects to Understand Stressor Interactions

***Eleanor L. Dearlove**, C.A.M. van Gestel, S. Loureiro, C. Svendsen, M. Lloyd, L Mugo-Kamiri, J.M. Petersen, E. Bessette, S. Edwards, Fang-Shiang Lim, P. Herren, Luis Hernández-Pelegrín, R Pienaar, H. Huditz, A Mostafaie, J. Pinto, A. Roman, C. Savio, A.R. Slowik, J. Takacs, J.K. Upfold, H. Hesketh*

CONTRIBUTED PAPERS. Thursday, 9:30 **DBI-13**

The Tick Cell Biobank – cell lines for research on biology and control of insects, ticks, and their associated microorganisms

***Catherine Hartley**, Jing Jing Khoo, Alistair C. Darby, Benjamin Makepeace, Lesley Bell-Sakyi*

CONTRIBUTED PAPERS. Thursday, 9:45 **DBI-14**

Near-complete bacterial genome insertion in a tick nuclear genome: evidence from tick cell lines and ticks

***Jing Jing Khoo**, Alexandra Beliavskaia, Catherine Hartley, Alaa Al-Khafaji, Grace Ward, Stuart Armstrong, Germanus Bah, Maria Kazimirova, Alistair C. Darby, Benjamin L. Makepeace, Lesley Bell-Sakyi*

CONTRIBUTED PAPERS. Thursday, 10:00 **DBI-15**

Monitoring of larval growth and persistence of *Bacillus thuringiensis* and *Clostridioides difficile* spores during bio-conversion of waste streams by black soldier fly larva (*Hermetia illucens*)

*Agnès Rejasse, Christophe Buisson, Ludovic Bridoux, Isabelle Poquet, Vincent Sanchis, **Christina Nielsen-Le Roux***

Contributed Papers Bacteria 4

Thursday, 8:30 – 10:15
Hlawka EI 9

Ecology and Regulation
Chairs: Omaththage Perera, Hannah Best

CHRIS LOMER AWARD Thursday, 8:30 **B-25**

Linocin M18 protein from the insect pathogenic bacterium *Brevibacillus laterosporus* isolates

***Tauseef K. Babar**, Travis R. Glare, John G. Hampton, Mark R. H. Hurst, Josefina Narciso, Campbell R. Sheen, Barbara Koch*

CONTRIBUTED PAPERS. Thursday, 8:45 **B-26-STU**

A novel approach for transforming *Bacillus thuringiensis* strain without the use of antibiotics

***Siyi Liu**, Dong Hwan Park, Minghui Wang, Jae Young Choi, Yeon Ho Je*

CONTRIBUTED PAPERS. Thursday, 9:00 **B-27**

Sporulation-independent Cry toxin production in Bt *kurstaki* strains

***Emilie Verplaetse**, Leyla Slamti, Didier Lereclus*

CONTRIBUTED PAPERS. Thursday, 9:15 **B-28**

Endophytic *Bacillus thuringiensis* strains: an unusual habitat and a potential biotechnological development

*Rosalina García-Suárez, Gabriela Espinoza-Vergara, Areli Cando-Narváez, Lucía Rodríguez-Maldonado, Ma. Cristina Del Rincón-Castro, **Jorge E. Ibarra***

CONTRIBUTED PAPERS. Thursday, 9:30 **B-29**

Plants recruit insecticidal bacteria to defend against herbivore attacks

*Wenyue Xu, Xiaoxiao Sun, Liang Mi, **Changlong Shu**, Jie Zhang, Lili Geng*

CONTRIBUTED PAPERS. Thursday, 9:45 **B-30**

Insights on *Pseudomonas protegens* ecology and bioinsecticidal properties

Luca Ruiu

CONTRIBUTED PAPERS. Thursday, 10:00 **B-31**

Rapid adaptation of *Bacillus thuringiensis* to alkaline environments via the L-lactate metabolism pathway regulated by CRP/FNR family regulator LtmR

***Qi Peng**, Jiaxin Qin, Hong Xu, Guiwei Kao, Fan Yang, Zhongqin Sun, Leyla Slamti, Shuyuan Guo, Fuping Song*

Contributed Papers Fungi 2

Thursday, 8:30 – 10:30
Fritz Paschke EI 10

Fungal Interaction with Hosts and beyond
Chairs: Jürg Enkerli, Enrique Quesada-Moraga

CONTRIBUTED PAPERS. Thursday, 8:30 **F-9-STU**

Symbiotic bacterial abundance and protective response of two-spotted spider mites against *Akanthomyces attenuauts* JEF-147

Gahyeon Song

CONTRIBUTED PAPERS. Thursday, 8:45 **F-10-STU**

Unraveling DNA methylation in the entomopathogenic fungus *Metarhizium pinghaense* NCHU-125

***Yi-Hsuan Li**, Ming-Ren Yen, Nian-Tong Ni, Fang-Min Chang, Yu-Shin Nai*

CONTRIBUTED PAPERS. Thursday, 9:00 **F-11**

Unraveling multi-organismal interactions in the mosquito holobiont to enhance microbial control

Jose Luis Ramirez

CONTRIBUTED PAPERS. Thursday, 9:15 **F-12**

The body-surface associated microbiome of soil-dwelling larvae of *Melolontha melolontha* increases resistance to its fungal pathogen *Beauveria brongniartii*

*Denise Baur, Noëmi Küng, Chiara Pedrazzini, Franco Widmer, **Jürg Enkerli***

CONTRIBUTED PAPERS. Thursday, 9:30 **F-13**

Influence of defense mechanisms of *Euschistus heros* (Hemiptera: Pentatomidae) during the infective process of *Metarhizium* spp.

*Aline Nunes da Silva, Janaína Brandão Seibert, Jhonattan Rodríguez Guerrero, Diego Magalhães, **Italo Delalibera Júnior***

CONTRIBUTED PAPERS. Thursday, 9:45 **F-14**

RNA-sequencing of entomopathogenic fungus-infected *Thrips plami* reveals change of host defense and homeostasis

*Yu Jin Jeong, Jong-Cheol Kim, Mi Rong Lee, Gahyeon Song, **Jae Su Kim***

CONTRIBUTED PAPERS. Thursday, 10:00 **F-15**

To be or not to be entomopathogenic depends on a mycovirus

*Fátima Rueda-Maillo, Inmaculada Garrido-Jurado, Enr**ique Quesada-Moraga***

CONTRIBUTED PAPERS. Thursday, 10:15 **F-16**

Zinc solubilization and organic acid production by the entomopathogenic fungus, *Metarhizium pingshaense* sheds light on its key ecological role in the environment

***C. M. Senthil Kumar**, Sharon D'Silva, R. Praveena, Anees Kaprakkaden, L. R. Athira Krishnan, M. Balaji Rajkumar, V. Srinivasan, R. Dinesh*

Refreshment Break
Thursday, 10:30 – 11:00
Foyer

SIP Membership Meeting
Thursday, 11:00 – 13:00
Lecture Hall EI 7

Lunch
Thursday, 13:00 – 14:00
Foyer

Pan-Division Symposium Viruses & DBI & Bacteria
Thursday, 14:00 – 16:00
Lecture Hall EI 7

Diseases in Invertebrates for Feed and Food: Global Perspectives
Chairs: Vera Ros, Helen Hesketh, Christina Nielsen-LeRoux

CDS VIRUSES, DBI, BACTERIA. Thursday, 14:00 **16-1**

Challenges of pathogens detection in insect mass rearing: Advances from the Insect Doctors program for improved diagnostic

Elisabeth Herniou

CDS VIRUSES, DBI, BACTERIA. Thursday, 14:30 **16-2**

Emerging Threats to Black Soldier Fly Farming in Africa

*Inusa J. Ajene, **Chrysantus M. Tanga**, Fathiya M. Khamis*

CDS VIRUSES, DBI, BACTERIA. Thursday, 15:00 **16-3**

Diseases in shrimp aquaculture

Kelly Bateman

CDS VIRUSES, DBI, BACTERIA. Thursday, 15:30 **16-4**

Microsporidia and protist parasites in reared insect hosts

***Edouard Bessette**, Bryony Williams, Nicolai V. Meyling*

- End of Scientific Program

Banquet
Arcotel Wimberger
Neubaugürtel 34-36, 1070 Vienna
Thursday, 18:30 – 00:00

Transportation to the venue and back via public transportation ticket. No transportation by the Conference organizers.

Oral Presentations

CONTRIBUTED PAPERS **B-1-STU**

Characterization of Cry1Aa binding through protein mutants in two lepidopteran pests

Dafne Toledo[1]*, Yolanda Bel*[1]*, Baltasar Escriche*[1]
[1]Laboratory of Biotechnological Control of Pests, BIOTECMED Institute, Genetics Department, University of Valencia, 46100-Burjassot, ES

Correspondence: dafne.toledo@uv.es

Bacillus thuringiensis (Bt) synthesizes pesticidal Cry proteins, which are expressed in transgenic crops and are the main active component of Bt-based formulations. Although the Cry proteins' mode of action is not fully characterized, their binding to midgut receptors is a necessary step for their efficacy. In this study, to gain insight into this step, site-directed mutagenesis was used to synthesize two Cry1Aa mutants previously described as possibly affecting cadherin receptor binding. The LC_{50} values were obtained after testing the toxicity of protein mutants and the wildtype Cry1Aa against *Ostrinia nubilalis* and *Grapholita molesta*. To characterize their toxicity loss, we performed oligomerization and ^{125}I-Cry1Aa binding assays with BBMVs. Furthermore, the ability of these proteins to bind to the cadherin fragment (from repeats 7 to 11/12) in both species was tested by dot blot assays. The results indicated that the mutations had different effects on toxicity depending on the insect species. Oligomerization and dot blot assays were similar in both lepidopterans. However, the competition assays using BBMVs showed different binding behaviours. Overall, the results suggest that although the mutated residues are involved in receptor binding, *O. nubilalis* and *G. molesta* cadherins may not be associated with the mutated regions of Cry1Aa.

CONTRIBUTED PAPERS **B-2**

VIP3Cb1 structural & functional studies: Insecticidal toxin from *Paenibacillus* spp. effective for controlling Lepidopteran insect pests in crops.

Tommi White[1]*, Meiying Zheng*[1]*, Timothy Rydel*[1]*, Michael Rau*[1]*, William Moar*[1]*, Todd Ciche*[1]*,* ***David Bowen***[1]*, Lucas Mckinnon*[1]*, James Fitzpatrick*[2]*, Adam Cutts*[3]*, Colin Berry*[3]
[1]Bayer Crop Science, Chesterfield, US; [2]Pharma Research & Early Development, Roche, Basel, CH; [3]School of Biosciences, Cardiff University, Cardiff, UK

Correspondence: david.bowen.bayer.com

Vegetative insecticidal proteins (VIPs) are a large class of insecticidal toxins that have a broad range of insecticidal activity. VIP3 is a class of toxins that have been used recently as insect-control traits in crops. Within the VIP3 toxin class, there are levels (A-D), which have been characterized functionally and more recently, structurally using cryoEM. Recently, a VIP3C toxin (VIP3Cb1) was discovered from *Paenibacillus* spp. Crop plants expressing Vip3Cb1 were effective in controlling major Lepidopteran pests. Sequence diversity was observed in the predicted receptor binding domains of Vip3Cb1, however partial resistance to VIP3Cb1 was observed in Vip3A-resistant colonies of *Spodoptera frugiperda* (fall armyworm) and *Helicoverpa zea* (corn earworm). The functional characterization and first structural studies of VIP3Cb1 utilizing cryoEM will be presented in both its protoxin and activated toxin form.

CONTRIBUTED PAPERS **B-3**

Analysis of Synergism between Extracellular Polysaccharide from *Bacillus thuringensis* and Insecticidal Protein

Meiling Wang[1]*, Bai Xue*[1,2]*, Tianjiao Ma*[1,2]*, Zeyu Wang*[1]*, Changlong Shu*[1]*, Lili Geng*[1]*,* ***Jie Zhang***[1]
[1]State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, CN; [2]College of Life Sciences, Northeast Agricultural University, Harbin, CN

Correspondence: zhangjie05@caas.cn

Bacillus thuringiensis (Bt) is the most widely used biopesticide worldwide and can produce several insecticidal crystal proteins and vegetative insecticidal proteins (Vips) at different growth stages. Microbial exopolysaccharides (EPSs) are a class of biological highmolecular-weight polymers that are synthesized and secreted into the culture medium during the growth and metabolism of microorganisms (e.g., bacteria, fungi, yeasts and microalgae). Our studies have revealed that 96.5% of strains in the Bt standard strain library are capable of producing EPS. Bt EPSs had universal synergistic effects on Cry1-type or Vip toxins against *Plutella xylostella* (L.)*, Spodoptera frugiperda* (J.E. Smith) and *Helicoverpa armigera* (Hübner). Bt EPS-HD270 and EPS-G03 exhibited synergistic activity with Vip3Aa through promotion of binding to BBMVs and protection from digestion by midgut protease. When EPS-HD270 and Vip3Aa11 protoxin were simultaneously fed to third-instar larvae, laser confocal microscopy observations revealed co-localization of the two compounds near the midgut wall, which aggravated the damage to BBMVs. The results indicated that synergistic activity with Bt toxins was an important function of Bt EPSs, which was much different from other *Bacillus* spp.

CONTRIBUTED PAPERS **B-4-STU**

Receptor Interactions of Vip3Aa Protoxin and Activated-Protein Structural Conformations in *Spodoptera exigua*

María Lázaro-Berenguer[1]*, Juan Ferré*[1]*, Patricia Hernández-Martínez*[1]
[1]Biotechnological Pest Control Laboratory, Institute of Biotechnology and Biomedicine (BIOTECMED), University of Valencia, Valencia, ES

Correspondence: maria.c.lazaro@uv.es

The Vip3Aa insecticidal protein, produced by *Bacillus thuringiensis*, has been effectively used in commercial Bt-crops to manage lepidopteran pests. Upon ingestion by larvae, the protoxin is proteolytically processed by midgut proteases into the activated protein and binds specifically to its receptors in the midgut epithelium, leading to insect mortality. CryoEM resolution of the trypsin-processed Vip3Aa protein unveiled structural remodelling of the N-terminal region during the transition from protoxin to the activated protein. This conformational change has been demonstrated to be crucial for the protein's toxicity against beet armyworm (*Spodoptera exigua*) larvae, a major global lepidopteran pest.
In this study, we investigated the relevance of the structural remodelling for the specific binding to midgut receptors. We conducted *in vitro* binding assays with radiolabelled proteins on Brush Border Membrane Vesicles (BBMV) from *S. exigua*, employing structural mutants that lock the protein in either its protoxin or activated conformation. Our findings indicate that both structural stages of the protein share receptors in the midgut epithelium, which implies flexibility in the mechanism of action. Moreover, *in vivo* competition assays revealed that Vip3Aa is able to bind to functional receptors in *S. exigua* both as protoxin and activated protein. Hence, i*n vivo*, either spontaneous structural shift upon protease cleavage or receptor-mediated remodelling could be occurring, showing the complex interplay between proteolytic processing, protein structure and receptor interactions.

CONTRIBUTED PAPERS **B-5**

Bacillus thuringiensis Vip3Aa structural changes upon proteolytic activation trigger receptor binding necessary for insect toxicity

*Oscar Infante[1], Isabel Gómez[1], Angel E. Pélaez[1], Luis A. Verduzco-Rosas[1], Rosalina García-Suárez[1], Zeyu Wang[2], Jie Zhang[2], Alejandra Bravo[1], **Mario Soberón**[1]*
[1]Instituto de Biotecnología, Universidad Nacional Autónoma de México, Cuernavaca, Morelos, MX; [2]Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, CN

Correspondence: mario.soberon@ibt.unam.mx

Bacillus thuringiensis (Bt) Vip3 insecticidal proteins form pores in the apical membrane of midgut cells from susceptible lepidopteran larvae causing the death of the insect. Vip3Aa protoxin is organized as a tetrameric-structure where each monomer is formed by five distinct domains. Upon proteolytic activation, Vip3 toxin undergoes a large conformational change forming a syringe like structure that is ready for membrane insertion and pore formation. Here we show that Vip3Aa protoxin does not bind to *Spodoptera frugiperda* brush border membrane vesicles (BBMV) in contrast to the activated toxin that binds specifically to BBMV proteins, suggesting that a structural change of Vip3Aa upon proteolytic activation is required for receptor binding. In agreement, Vip3Aa protoxin showed low toxicity to Sf9 cells in contrast to the activated toxin. In contrast, both Cry1Fa protoxin and activated toxin were toxic to Sf9 cells. By analyzing the binding of different Vip3Aa overlapping peptides to *S. frugiperda* BBMV, a domain III region containing a Vip3Aa binding epitope was identified. In addition, analysis of amino acid residues that become exposed upon activation of Vip3Aa and mutagenesis of those exposed residues, allowed us to identify two structurally adjacent loop regions that form a structural binding epitope which plays an important role in receptor binding and toxicity against *S. frugiperda* larvae. Our results show that proteolytic activation of Vip3Aa triggers receptor binding necessary for toxicity.

CONTRIBUTED PAPERS **B-6-STU**

Interactions of vegetative insecticidal proteins with target membranes

***Adam Cutts**[1], Paola Borri[1], William Moar[2], Tommi White[2], Colin Berry[1]*
[1]Cardiff University, Cardiff, UK; [2]Bayer Crop Science, Chesterfield, MO, US

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Vegetative insecticidal proteins (Vip3s), expressed by *Bacillus thuringiensis* (Bt) display insecticidal activity against lepidopterans and, as such, have been used in the control of agricultural pests. Vip3 use has become more widespread as an alternative to Cry proteins, another family of insecticidal proteins of Bt origin, due to the emergence of resistance against Crys. Vip3s currently show no cross-resistance with Cry proteins in target organisms, making them a potentially potent and viable substitute.
Although used successfully, little is understood about Vip3 mode of action, any interactions with components of target membranes, and how physiological or biochemical changes in insects leads to resistance, which is beginning to arise as an issue. Novel microscopy and electrophysiological techniques are being used to elucidate further details of Vip3 activity.
Droplet interface bilayer systems allow for determination of Vip3 ion preference and/or specificity when inserted into target membranes, with concurrent electrophysiological analysis to characterise ion movements. Droplet hydrogel bilayer systems, in combination with total internal reflectance fluorescence (TIRF) microscopy, have allowed direct imaging of ion efflux through pores formed by Vip3s. Cutting-edge interferometric gated off-axis reflectance (iGOR) microscopy offers label-free imaging of Vip3 particles in bilayer systems. iGOR is being used to understand how insertion of Vip3 disrupts local membrane environments and allows for assessment of how Vip3 insertion and subsequent membrane perturbation contributes to Vip3 toxicity Molecular modelling is being used to demonstrate interactions of Vip3 with membrane components, as well as support mutagenic studies.

CONTRIBUTED PAPERS **B-7**

The folding-cane model to explain major conformational changes of *Bacillus thuringiensis* Cry1Ab required for membrane insertion and toxicity

*Sabino Pacheco[1], Jorge Sánchez[1], Isabel Gómez[1], Blanca García[1], Mario Soberón[1], **Alejandra Bravo**[1]*
[1]Instituto de Biotecnología , Universidad Nacional Autónoma de Mexico, , MX

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Pore forming toxins rely on oligomerization for membrane insertion to kill their targets. *Bacillus thuringiensis* produces insecticidal Cry-proteins that insert into the larval midgut cells forming pores that kill the insect larvae.
The structural changes involved in membrane insertion of these proteins remain unsolved. The most widely accepted model for membrane insertion, the 'umbrella model', proposed that the α-4/α-5 hairpin of Domain I swings away and is inserted into the membrane. However, some data contradict this model. To determine the topology of Cry1Ab in the membrane, disulfide bonds linking α-helices of Domain I were introduced to restrict their movement. Disulfide bonds between helices α-2/α-3 or α-3/α-4 lost oligomerization and toxicity, indicating that movement of these helices is needed for insecticidal activity. By contrast, disulfide bonds linking helices α-5/α-6 did not affect toxicity, which contradicts the 'umbrella model'. Additionally, Föster resonance energy transfer-closest approach analyses measuring distances from different points in the toxin to the membrane plane and collisional quenching assays, analyzing the protection of specific fluorescent-labeled residues to the soluble potassium iodide quencher in the membrane inserted state were performed. Overall, the data show that Domain I from Cry1Ab undergo major conformational changes during its membrane insertion, where the N-terminal region (helices α-1 to α-4) participates in oligomerization and toxicity. These data break a paradigm, showing a new 'folding-cane model', which better explains the structural changes of Cry toxins during their insertion into the membrane.

CONTRIBUTED PAPERS **B-8-STU**

The first pore structure of the independent and insecticidal Bacterial Exotoxin B protein Vpb4

***Raymond Wirawan**[1], Bradley Spicer[1], Oliver Castell[2], Charles Bayly-Jones[1], Chris Lupton[1], David Jamieson[2], Hannah Baird[2], Dafydd Jones[2], Lainey Williamson[2], Husam Sabah Auhim[2], Hannah Best[2], Hari Venugopal[1], Colin Berry[2], Michelle Dunstone[1]*
[1]Monash Biomedicine Discovery Institute, Clayton, AU; [2]Cardiff University, Cardiff, UK

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The Bacterial Exotoxin B family is composed of pore-forming proteins that bind to the cell surface and perforate the cells, allowing the transport of a secondary component into the cell to confer toxicity. While most family members are toxic to humans and livestock (e.g., PA from anthrax or CdtB toxin), one class called the Vpb, was discovered to be lethal against insect species. The Vpb4 subclass, in particular, was found to exhibit larvicidal activity against the economically important pest Western Corn Rootworm. Importantly, the protein can function in the absence of any A component, making it a unique member of the class

and family. The precise molecular mechanism regarding how Vpb4 targets and kills, however, remains limited.
Here, through single-particle cryo-electron microscopy, we elucidate the first pore structure of the Vpb4 proteins at 3.07 Å resolution. The structure revealed several distinguishing features compared to the typical Bacterial Exotoxin B pores, including the absence of the highly conserved phenylalanine clamp and the overall neutral charge of the pore lumen. Through electrophysiology experiments, we also showed that the Vpb4 pore can insert into lipid membranes and modulate conductivity akin to proteins with a direct mechanism of killing, rather than an ability to transport another toxin. Collectively, the Vpb4 pore structure highlights important molecular features of the protein as a single-component insecticidal toxin for future application in the agricultural industry.

CONTRIBUTED PAPERS **B-9**

Development and characterization of western corn rootworm, *Diabrotica virgifera virgifera* LeConte Resistant to Mpp75Aa1.1 from *Brevibacillus laterosporus* and Vpb4Da2 from *Bacillus thuringiensis*

William Moar[1], *Chitvan Khajuria*[1], *Kaylee Miller*[1], *Michael Pleau*[1], *Brent Werner*[1], *Yong Yin*[1], *Paula Price*[1], *Juan Ferre*[2], *Graham Head*[1]
[1]Bayer Crop Science, Chesterfield, US; [2]Institute of Biotechnology and Biomedicine (BIOTECMED), Department of Genetics, Universitat de València, Burjassot, ES

Correspondence: william.moar@bayer.com

Transgenic maize expressing *Bacillus thuringiensis* (Bt) proteins has been rapidly adopted on farms across the Midwestern U.S. Corn Belt to control western corn rootworm (WCR), *Diabrotica virgifera virgifera* LeConte and other corn rootworm (CRW) species. However, at least some resistance has been reported to all five Bt proteins currently used to control WCR, emphasizing the need for alternative management tools. The Mpp75Aa1.1 protein from *Brevibacillus laterosporus* and the Vpb4Da2 protein from *B. thuringiensis* are two beta pore-forming proteins with high efficacy against WCR that do not have cross resistance to any current commercial Bt protein. This presentation will discuss the development and characterization of resistance to Mpp75Aa1.1 and Vpb4Da2 by WCR.

CONTRIBUTED PAPERS **B-10**

Characterization of a Caenorhabditis elegans strain highly resistant to Cry14A family proteins

YouMie Kim[1], *Thanh-Thanh Nguyen*[1], *Takao Ishidate*[1], *Daniel Durning*[1], *Ozkan Aydemir*[1], *Craig Mello*[1], *Yan Hu*[2], ***Raffi Aroian***[1]
[1]UMASS Chan Medical School, Worcester, US; [2]Worcester State University, Worcester, US

Correspondence: raffi.aroian@umassmed.edu

Bacillus thuringiensis (Bt) Cry proteins that kill insect pests have been produced in transgenic crops such as corn, cotton, and soybean that have been planted on a cumulative total of 1.5 billion hectares from 1996 to 2022. Hundreds of Cry proteins in >50 different families have been characterized and we found that some Cry proteins (*e.g.*, Cry5Ba, Cry14Aa), related by sequence and structure to those used to combat insects, can kill nematodes. We have studied Cry proteins extensively against human and animal gastrointestinal nematode (GIN) parasites and also found that Cry5Ba, when expressed in transgenic tomato roots, provided control over infection by the root-knot plant-parasitic nematode (PPN) *Meloigoyne incognita*.This result suggested that transgenic plants expressing a nematode-active Cry protein might provide protection against endoparasitic PPNs. With this goal in mind, Cry14Ab was reported to be expressed in transgenic soybean and significantly impaired the reproduction of by the PPN soybean cyst nematode. Transgenic Cry14Ab soybean plants, which have received regulatory approval from the US Environmental Protection Agency (EPA) and Food and Drug Administration (FDA), can fill an important gap in providing protection against *H. gycines*. To date, however, whether or not nematodes could develop high-level resistance to Cry14A family proteins and, if so, via what pathways, was not known. Since this question is critically important with regards to deployment of transgenic crops expressing Cry14A family proteins on a large scale, here we describe the isolation, identification, and characterization of a newly identified *Caenorhabditis elegans* strain and pathway that mutates to resistance to Cry14A family proteins, albeit with a high fitness cost.

CONTRIBUTED PAPERS **B-11-STU**

Lack of tolerance development following sublethal Cry1 protein exposure in *Spodoptera exigua* (Hübner)

Sandy Valdiviezo-Orellana[1], *Patricia Hernández-Martínez*[1], *Baltasar Escriche*[1]
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The insecticidal proteins derived from *Bacillus thuringiensis* (Bt) have been effectively employed in controlling lepidopteran pests, notably in transgenic crops targeting *Spodoptera* species. However, concerns have arisen regarding the long-term efficacy due to the development of tolerant and resistant insect populations. Prior research suggested that immune priming might impact Bt tolerance, but the specific effects of subsequent exposure to isolated Bt proteins remain unclear. This study aimed to assess whether prior exposure of neonate *Spodoptera exigua* larvae to Cry1Ab and Cry1Ca toxins would heighten their toxicological response upon subsequent exposure and whether such effects would extend to their offspring. Cry1Ab and Cry1Ca were selected for their distinct effects on *S. exigua*, with Cry1Ab causing growth inhibition and Cry1Ca leading to mortality and growth inhibition. However, pre-exposure did not significantly alter larval weight or growth inhibition compared to controls, with consistent findings in transgenerational analyses. These results suggest that immunological priming may not substantially impact the efficacy of Bt proteins. This study provides novel insights into *S. exigua*'s response to Cry proteins, indicating limited potential for Bt tolerance development in transgenic crops.

CONTRIBUTED PAPERS **B-12-STU**

Knockdown of *Egfr* and *Stmn4* by using an efficient oral feeding RNAi system increases the susceptibility of *Spodoptera frugiperda* larvae to Cry1F protein

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Crystal (Cry) proteins produced by *Bacillus thuringiensis* (Bt) efficiently control many lepidopteran pests. However, the low efficiency of RNA interference (RNAi) limited the study of their defense mechanism to Cry proteins in the midgut. *Epidermal growth factor receptor* (*Egfr*) and *stathmin 4* (*Stmn4*) genes regulate stem cell proliferation and cell cycle in *Drosophila*. In this study, we showed that *SfEgfr* and *SfStmn4* both highly expressed in the midgut of 4th instar *S. frugiperda* larvae. *SfEgfr* and *SfStmn4* dsRNA synthesized in vitro showed better silencing efficiency compared to the expressed in *Escherichia coli*, especially

when it was combined with star-polycation complex (SPc) nanoparticles. The highest silencing efficiency (over 75%) of those two genes were achieved at the concentration of 25 µg synthesized dsRNA-SPc per gram diet for 48 hours. *SfEgfr* and *SfStmn4* were significantly unregulated in midgut after treating Cry1Fa for 48 h. Pretreatment with dsRNA for 48 h increased the mortality of larvae treated with Cry1Fa for more than 40%. Silencing of these two genes dampened intestinal stem cell proliferation in the midgut after Cry1Fa treatment, which explains the reason why the mortality increased. This study provided an efficient oral feeding RNAi system for silencing midgut genes in lepidopteran pests, and demonstrated that *SfEgfr* and *SfStmn4* play a crucial role in the midgut defense response to Bt Cry proteins.

CONTRIBUTED PAPERS **B-13**

Impacts of gene introgression from invasive *Helicoverpa armigera* into native *Helicoverpa zea* on the efficacy of transgenic crops in the USA.

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The establishment of invasive species populations can threaten the ecological balance in naïve habitats and impact agricultural production practices. The old-world bollworm (OWBW), *Helicoverpa armigera*, and the corn earworm (CEW), *H. zea*, were geographically separated prior to the 2013 report of OWBW invasion into South America. Introgression of OWBW specific cytochrome P450 337B3 (CYP337B3) gene into CEW was repeatedly detected across South America and the Caribbean. Two hybrids were documented among samples collected in Texas in 2019. In this study, screening insects collected across the USA detected high allele frequencies of insects with the OWBW-specific CYP337B3 marker. Nucleotide sequencing of the CYP337B3 gene identified CYP337B3v2 and CYP337B3v6 alleles within the CEW samples analysed. Based on prior data for distinct phylogeographic origins of CYP337B3v2 and CYP337B3v6 alleles, our results indicate that USA populations were derived from two different introductions. Specifically, a novel origin based on restricted distribution of v6 allele to Ghana in West Arica, and possible South American or Caribbean origin of the v2 allele. One of the 1618 individuals screened also carried a ribosomal RNA internal transcribed spacer 1 (ITS1) derived from OWBW. Local selection pressures at the Olathe location imposed by repeated pyrethroid exposures are likely attributed to the prevalence of CYP337B3, where control practices hasten the accumulation of phenotypic resistance by adaptive introgression. High frequencies of CYP337B3 alleles in the USA populations of CEW indicate the possibility of introgression of OWBW genes conferring resistance to plant-incorporated *Bacillus thuringiensis* proteins into CEW. The genes introgressed from OWBW may continue to impact CEW management tactics across the Americas.

CONTRIBUTED PAPERS **B-14**

Identifying resistance alleles to Bt corn in European corn borer with multiplexed targeted sequencing

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The European corn borer (ECB, *Ostrinia nubilalis*) is a $1 billion pest in the US managed primarily by transgenic maize producing insecticidal proteins from *Bacillus thuringiensis* (Bt corn). Practical resistance to Bt corn producing the Cry1F protein originally evolved in 2018 in Nova Scotia (Canada), and reduced susceptibility to Cry1Ab and Cry1A.105 proteins has been detected in Canadian ECB populations. Previous work detected six allelic polymorphisms in the ABCC2 gene unique to ECB larvae with the field-evolved Cry1F resistant phenotype. We now report on the use of a highly multiplexed targeted sequencing approach (HiPlex) in identifying specific alleles tightly associated with the Cry1F resistant phenotype and efforts to routinely detect these alleles in field ECB samples. The results from this study identify a candidate Cry1F resistance allele and support the use of HiPlex in field monitoring of ECB resistance to Cry1F corn.

CONTRIBUTED PAPERS **B-15**

New paralogs of the *Heliothis virescens* ABCC2 transporter as potential receptors for Bt Cry1A proteins

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The ATP-Binding cassette (ABC) transporters are a superfamily of membrane proteins. These active transporters play a role in exporting various substances such as xenobiotics. ABC transporters from subfamily C (ABCC) have also been described as functional receptors for different insecticidal proteins from *Bacillus thuringiensis* (Bt) in several lepidopteran species. Numerous studies have explored the relationship between the ABCC2 transporter and Bt Cry1 proteins. Although other ABCC transporters sharing structural and functional similarities have been described, little is known of their role in the mode of action of Bt proteins. To date, only the ABCC2 transporter and its interaction with Cry1A proteins have been studied in *Heliothis virescens*. In this study, we searched for paralogs to the *ABCC2* gene in *H. virescens*, and identified two new ABC transporter genes, ABCC3 and ABCC4. Furthermore, we have characterized their gene expression in the midgut and their protein topology, and compared them with that of ABCC2. Finally, we discuss their possible interaction with Bt proteins through protein docking analysis.

Genetics of insect resistance to Vip3Aa

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The VIP (vegetative insecticidal proteins) toxins from *Bacillus thuringiensis* kill insects via different pathways compared with the Cry toxins. Vip3Aa is deployed in transgenic crops as a backup against pest evolution of Cry toxin resistance, and is currently effective against the fall armyworm, *Spodoptera frugiperda*. However, Vip3Aa-resistant strains of several pest species are now known. Two different mutations associated with resistance to Vip3Aa have been reported from lab-selected strains of invasive *S. frugiperda* from China. One is a change in the promoter sequence of SfMyb, a transcription factor that apparently controls the expression of protein binding targets of Vip3A in the midgut. Another is insertion of a transposable element that disrupts the coding sequence of SfCHS2, the chitin synthase responsible for chitin production in the peritrophic matrix in the midgut. Knockouts of the chitin synthase gene conferred resistance to Vip3Aa in *S. frugiperda* and two other lepidopteran pests. We expect that mutations conferring *S. frugiperda* resistance to Vip3Aa occur in loci other than *SfMyb* and *SfCHS2*. Anticipating and managing Vip3Aa resistance is important for sustainable pest control, especially with field-evolved resistance to Cry toxins conferred by a dominant mutation affecting tetraspanin and associated with increased protease activity.

Bacillus thuringiensis serovar kurstaki (Btk) Strains for Industrial Production in wheat bran based medium: Insights from Genomic Exploration and Nutritional Optimization

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Bacillus thuringiensis serovar kurstaki (Btk) strain, Lip, isolated from Lebanese soil produce bipyramidal and cubic crystals of various δ-endotoxins, with higher toxicity against *E. kuehniella* larvae than HD-1. Within the framework of the IPM-4-Citrus project (MSCA RISE, No. 734921, 2017-2023), which aims to optimize the cultivation of these strains using a wheat bran-based medium (WB), a whole genome sequencing (WGS) approach was utilized first to explore all genomic aspects of *Btk* Lip. Lip strain was found to possess 11 plasmids, with pLip300 carrying genes encoding delta-endotoxins including Cry1Ab, 2Cry1Ac, Cry1Aa, Cry1Ia, Cry2Aa, and Cry2Ab.
For industrial production, WB byproduct was chosen for its cost effectiveness and nutrient richness. WB was sorted into four different sizes: class 1 (>850 µm), class 2 (500 µm Btk, although the hemicellulosic fraction was also consumed at a rate of 0.15-0.2 g/gWB. Subsequently, nutritional constraints were identified with a focus on five elements (C, H, O, N, S). Notable differences in nitrogen mass balance were seen. Nitrogen emerged as a limiting nutrient at the flask scale. The C/N ratio proved to be a reliable indicator for assessing culture progress in flasks

Bacillus thuringiensis Spores and Cry Toxins Act Synergistically to Expedite Colorado Potato Beetle Mortality: Mechanisms, Application and Perspectives

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The bacterial biopesticide *Bacillus thuringiensis* (Bt) enters the insect host via the mouth and must thwart gut-based defences to make its way into the body cavity (haemocoel) and establish infection. We sought to uncover the main antibacterial defences of the midgut and the pathophysiological features of Bt in a notable insect pest, the Colorado potato beetle *Leptinotarsa decemlineata*. Exposing the beetles to both Bt spores and their Cry3A toxins (crystalline δ-endotoxins) via oral inoculation led to higher mortality levels when compared to either spores or Cry3A toxins alone. Within 12 h post-exposure, Cry3A toxins caused a 1.5-fold increase in the levels of reactive oxygen species and malondialdehyde (lipid peroxidation) within the midgut – key indicators of tissue damage. When Cry3A toxins are combined with spores, gross redox imbalance and 'oxidation stress' is apparent in beetle larvae. The insect detoxification system is activated when Bt spores and Cry3A toxins are administered alone or in combination to mitigate toxicosis, in addition to elevated mRNA levels of candidate defence genes. The presence of bacterial spores and/or Cry3A toxins coincides with subtle changes in microbial community composition of the midgut, such as decreased *Pseudomonas* abundance at 48 h post inoculation. Both Bt spores and Cry3A toxins have negative impacts on larval health, and when combined, likely cause metabolic derangement, due to multiple tissue targets being compromised. Thus, identifying the activity of various Bt virulence factors in the midgut, as well as understanding the involvement of host defense systems, is necessary for further enhancing biological products. The study also considers the potential of RNA interference and modified silicon dioxide nanoparticles to enhance the efficiency of Bt biopesticides against Colorado potato beetle.
This work was supported by the Russian Science Foundation (grant number 22-16-20031) and Governments of Novosibirsk region (№ p-4).

The use of *Bacillus thuringiensis* to control root-knot nematodes

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The use of biological pesticides such as *Bacillus thuringiensis* (Bt) arises from the global imperative to increase crop protection while preserving the environment. Bt is a Gramm positive bacterium that can produce pesticidal proteins which accumulate in parasporal crystals along the sporulation process. So far, Bt proteins belonging to 8 different families (App6, Cry5, Cry12, Cry13, Cry14, Cry21, Cry31, and Xpp55) have been reported to have activity against nematodes, the predominant organisms in many soil ecosystems.
Root-knot nematodes (RKN) are obligate plant parasitic nematodes distributed worldwide that parasitize roots, inflicting severe damage on plants, leading to global substantial yield losses with extensive economic repercussions. In this work, we have studied the toxicity of the

Cry5, Cry21, App6, and Xpp55 Bt proteins against the RKN *Meloidogyne incognita* and *M. javanica*, species found in tropical, subtropical, and temperate regions worldwide. *M. incognita* is probably the most widely distributed RKN species and *M. javanica* is considered a major agricultural pest in many countries. The *in vitro* results show that the four tested Bt proteins, when solubilized, were highly toxic for both species. To assess if Bt strains producing nematicidal crystal proteins can be used for controlling *Meloidogyne* spp. in the fields, *in planta* assays with cucumber or with tomato plants were carried out, using two wild Bt strains producing Cry5 or a combination of Cry5 and App6 proteins. The plants were watered twice with spore+cristal suspensions (on day 3 and day 7, after seedling planting) and after the second irrigation, the plants were also infested with *M. javanica* J2. Parameters such as egg masses in roots, emerged J2, and the dry foliar weight were evaluated after 8 weeks. The results showed that the nematicidal activity was plant-dependent and much lower than expected, highlighting the crucial role of Bt proteins delivery for effective control of RKN in the field.

CONTRIBUTED PAPERS **B-20**

Bacillus thuringiensis Cry proteins - an arsenal of anti-parasitics

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Bt Crystal proteins have until recently been used commercially exclusively for control of insect pests and account for 90% of the biopesticide market. Our group has spearheaded efforts to bring Cry proteins into clinical/commercial use against gastrointestinal parasitic nematodes (GINs). Gastrointestinal nematode (GIN) parasites are critical targets in both human health, with >1.5 billion people infected, and animal health (*e.g.*, sheep, pigs, horses, dogs, cattle) for which nematode parasites are nearly ubiquitous. In addition, GIN resistance in human and animal health to currently used drugs is increasing. Thus, there are significant opportunities for Cry proteins to make a difference as anthelmintics in global and animal health. We developed a new fermentable production and delivery method for Cry proteins suitable for therapeutic oral dosing against GINs, which we call IBaCC (inactivated bacteria with cytosolic crystals). Our published studies to date have focused on Cry5Ba IBaCC as a therapeutic against GIN infections in rodents, sheep, horses, and pigs. We have now expressed at least five additional Cry proteins in the IBaCC system. We are performing in vitro and in vivo curative studies of all six Cry IBaCCs against diverse GIN parasites ranging from hookworms to roundworms to whipworms. Here we will present an update on the efficacy of multiple Cry proteins in vitro and in vivo against these parasitic nematodes with the aim of eventually deploying combination Cry anthelmintic products.

CONTRIBUTED PAPERS **B-21**

Building an insecticidal protein gene library: deep expansion

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Numerous insecticidal protein genes from *Bacillus thuringiensis* (Bt) and other species were identified, 1103 of which were published on BPPRC database, and this number continues to rise. These genes are key components in the development of transgenic crops and pesticides. However, the bioassay data of their encoded insecticidal proteins is very limited, with many of them lacking targets or accurate activity assessments. Concurrently, the agricultural pests requiring control encompass a broad spectrum of species, including those in Insecta as well as nematodes, mites, and protozoa. Obtaining complete bioassay activity data of available insecticidal proteins will demonstrate their academic value and contribute to further development and utilization. In this study, we are building a physical insecticidal protein gene library, comprising both publicly available insecticidal genes and unreleased novel genes. We utilize the dual host systems, *E.coli* and/or Bt, for the preservation and expression of these genes. This library would be shared globally for free. Currently, 702 insecticidal genes, including 262 holotype genes (those name ending with the number 1), were cloned and 360 of them were expressed. We have evaluated this library and found some novel pesticidal proteins exhibiting significant toxicity against Lepidopteran insects, aphids or nematodes. This implies that this gene library has enormous potential to reveal their inherent activity by global test. Meanwhile, we encourage interested colleagues to participate in to accelerate the library construction.

CONTRIBUTED PAPERS **B-22**

Selecting a customised Crybody for the Mediterranean fruit fly *Ceratitis capitata.*

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Currently, there are 760 different natural 3D-Cry toxins produced by *Bacillus thuringiensis* described in the literature with a publicly available sequence (https://www.bpprc-db.org/database/). 3D-Cry toxins are mainly known for being toxic to insects and characterised for being highly specific toward their targets. While this specificity is beneficial for safety and the environment, it could represent a disadvantage when targeting a specific insect is needed. Finding natural toxins active toward certain species it could be sometime very challenging, involving a high workload.

The *in vitro* modification of natural 3D-toxins to redirect the activity toward a different insect is a realistic approach to solve this problem (DOI: 10.3390/toxins12090600), but the workload of bioassaying each mutant is still high and represent a very inefficient process, as most of the mutants are not stable or do not bind to a receptor in the host, so they cannot exert toxicity. To solve this, we have developed a strategy, based in the phage display technology of a library of what we call Crybodies (a chimeric between a Cry toxin and an antibody), that selects only those mutants able to bind to gut proteins of the target insect, reducing the number of candidate mutants to be bioassayed. We tested our technology in *Aedes aegypti* and selected active Crybodies almost as potent as the natural toxins (DOI: 10.1038/s41598-017-09384-x).

In the study presented here we test our methodology with the Mediterranean fruit fly, *Ceratitis capitata*, a significant world-wide distributed fruit pest with no active 3D-Cry toxin known. We selected 5 phage displaying Crybodies recognising *C. capitata* gut proteins, we cloned and heterologous-expressed them and tested if they were active. Our results shows that the Cry1Aa13-C4 Crybody shows a significant activity toward *C. capitata* adults, higher than the other selected Crybodies and the negative controls. This work shows once again the strength of our technology.

CONTRIBUTED PAPERS **B-23**

How pest resistance to *Bacillus thuringiensis* helps to improve bioinsecticides?

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Reduction or loss of virulence, morphological differentiation of bacteria *B. thuringiensis* when cultivated on an industrial scale, as well as the formation of pest populations resistant to biological insecticides, is a relevant area of research that requires the attention of scientists. Bacteria *Bacillus thuringiensis* (Bt) is one of the most common sources of biopesticide and gene modified crops used for pest management in the world. Bt produce a wide range of Cry-proteins and other virulence factors against insects, mites and nematodes. Many cases of insect's resistance to Bt toxins were registered last years. We experimentally selected a *G. mellonella* line over 40 generations for resistance against Bt. The *B. thuringiensis* subsp. *galleriae* infection in susceptible and resistant populations of wax moth larvae was investigated to gain further insight into the "arms race" between *Bt* virulence and insect defences. We found that a sub-population of highly virulent *B. thuringiensis* could survive in the resistant insects (with enhanced immune defences) by disrupting the midgut microbiome and switching rapidly to a necrotrophic strategy, prior to sporulation in the cadaver (Grizanova et al., 2023). Arm race between virulence factors of Bt and insect resistance is interesting example of coevolution in the field. Sequential passage, and isolation, of Bt through resistant insects for developing highly virulent strains will be discussed in context of applied biotechnology for improvements of biological preparations and their application. This work was supported by the Russian Science Foundation (grant number 24-16-00113).

CONTRIBUTED PAPERS **B-24**

Risk Assesment of New Btk Isolates BLB1 and LIP Biopesticides via Toxicity Assays on Lab Animals

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Bacillus thuringiensis (Bt), gram positive soil bacteria has been widely employed as a biopesticide against lepidopteran pests. In this research, two new *Bacillus thuringiensis* subsp. *kurstaki* (Btk) isolates BLB1 (Tunisien) and LIP (Lebanon) were formulated as dry products at JKI and to evaluate their biosafety, toxicity assays were conducted on lab animals (rabbit and guinea pig) in accordance with European criteria ISO and OECD guidelines. The final goal was to determine the risk for farmers/exposed populations and the impact on health. The experiments followed a comprehensive Action Plan (AP), with similar reference product on the market (DELFIN-WG) and a control formulation without active ingredients, with a focus on animal welfare considerations. Eye irritation test was performed on rabbits using both acute and subacute assays and results were evaluated using the "Maximum Mean Total Scores (MMTS)" formula. For the dermal irritation test, three rabbits/product (single exposure) were utilized, and the risk of the products was assessed based on the Primary Irritation Index (PII). The sensitization test was carried out on guinea pigs by intradermal administration in subcontracted company Kobay lab animal facilities. Protocols were approved in advance by the Ethical Committee of Institut Pasteur Tunis. Consequently, comparing the effects of BLB1 and LIP formulation to DELFIN-WG reference product, no mortality or abnormal clinical observations noted during the external toxicity tests. No significant reductions in weight or in feed and water intake was observed. The only necessity for repetition arose in the skin sensitization test due to a small number of mortalities observed in the negative control group. The preliminary evaluation of human risk from the new Btk-based formulations is promising, showing non-irritating effects on eyes and skin.

CHRIS LOMER AWARD CONTRIBUTED PAPERS **B-25-~~STU~~**

Linocin M18 protein from the insect pathogenic bacterium *Brevibacillus laterosporus* isolates

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Brevibacillus laterosporus (*Bl*) is a Gram-positive and spore-forming bacterium. Insect pathogenic strains have been characterised in New Zealand and two isolates, *Bl* 1821L and *Bl* 1951, are under development for use in biopesticides. However, growth in culture is sometimes disrupted, affecting mass production. Based on previous work, it was hypothesised that Tectiviridae phages might be implicated. While investigating the cause of the disrupted growth, electron micrographs of crude lysates showed structural components of putative phages including capsid and tail-like structures. Sucrose density gradient purification yielded a putative self-killing protein of ~30 kDa. N-terminal sequencing of the ~30 kDa protein identified matches to a predicted 25 kDa hypothetical and a 31.4 kDa putative encapsulating protein homologs, with the genes encoding each protein adjacent in the genomes. BLASTp analysis of the homologs of 31.4 kDa amino acid sequences shared >98.6% amino acid identity to the Linocin M18 bacteriocin family protein of *Brevibacterium* sp. JNUCC-42. Bioinformatic tools including AMPA and CellPPD defined that the bactericidal potential originated from a putative encapsulating protein. Antagonistic activity of the ~30 kDa encapsulating protein of *Bl* 1821L and *Bl* 1951during growth in broth exhibited bacterial autolytic activity. LIVE/DEAD staining of Bl 1821L cells after treatment with the ~30 kDa encapsulating protein of *Bl* 1821L substantiated the findings by showing 58.8% cells with the compromised cell membranes as compared to 37.5% cells in the control. Furthermore, antibacterial activity of the identified proteins of *Bl* 1821L was validated through gene expression in a Gram-positive bacterium *Bacillus subtilis* WB800N.

CONTRIBUTED PAPERS **B-26-STU**

A novel approach for transforming *Bacillus thuringiensis* strain without the use of antibiotics

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Bacillus thuringiensis (Bt) is widely used as a microbial pesticide due to its insecticidal proteins. However, genetically modified Bt strains that rely on antibiotic resistance genes can pose environmental risks. Therefore, this study aimed to develop a new screening approach using the sporulation mechanism of Bt instead of antibiotic resistance genes. First, the *spoOA* gene was knocked-out by homologous recombination to create a sporulation-deficient Bt strain. The plasmid carrying the *cry* and *spoOA* genes was then transformed into the sporulation-deficient Bt

strain by electroporation to restore its ability to sporulate. Through a heat shock process, Bt strains that have introduced the plasmid and sporulated could be screened from those that have not. These results indicate the possibility of using sporulation as a new selection pressure which could be used as an alternative to antibiotic resistance genes.

CONTRIBUTED PAPERS **B-27**

Sporulation-independent Cry toxin production in Bt *kurstaki* strains

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In most *Bacillus thuringiensis* (Bt) strains, the insecticidal *cry* genes are transcribed during sporulation by RNA polymerase containing sigma factors SigE or SigK. Consequently, the insecticidal crystal is formed in the mother cell concomitantly with the spore. Some strains, notably the *kurstaki* HD1, also produce insecticidal toxins that are liberated in the extracellular medium, specifically the Vip3A and Cry1I toxins. Their regulation was unknown until we identified VipR, an autoregulated transcriptional regulator that activates the expression of the *vip3A* toxin gene at the onset of the stationary phase. The determination of a putative VipR-binding box extended the VipR regulon to 7 others genes or operons in the HD1 strain: the *cry1I* and *cry2Ab* genes and 4 putative operons containing *cry1A* or *cry2Aa* genes. Interestingly, a VipR-box was also identified on the pHT73 plasmid of the *kurstaki* HD73 strain, closely related to the HD1 but not carrying *vipR*. The VipR-box is present in the promoter region of a putative N-acetylmuramoyl-L-alanine amidase-encoding gene located upstream the *cry1Ac* gene. A functional *vipR* gene was introduced in the HD73 strain and, using ß-galactosidase assays and RT-PCR, we demonstrated that VipR activates the transcription of the amidase and *cry1Ac* genes, thus forming a single transcriptional unit. Under these conditions, Cry1Ac was produced from the entry into and throughout the stationary phase of growth and larger bipyramidal crystals were formed. Cry1Ac was also produced during stationary phase under the control of VipR in a sporulation-deficient (Spo^-) HD73 strain, suggesting that the VipR regulon can be expressed in a Spo^- HD1 strain. Indeed, we showed that a sporulation-deficient HD1 still retains the ability to form crystals. Altogether, our results provide evidence of another example of sporulation-independent Cry toxin production in Bt *kurstaki* strains.

CONTRIBUTED PAPERS **B-28**

Endophytic *Bacillus thuringiensis* strains: an unusual habitat and a potential biotechnological development.

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Isolation of *Bacillus thuringiensis* (Bt) strains from inner plant tissues have been reported for a diversity of crop plants such as maize, cotton, soybean, coffee, wheat, citrus, common beans, among others. This endophytic phenomenon has been induced by inoculating spore-crystal complexes of genetically marked strain into the plant's rhizosphere in cotton, soybean, and in this work, in common bean and *Arabidopsis thaliana*. Bacterial translocation has been proven by the re-isolation of the marked strain from leaves and stems of translocated plants. Insecticidal activity of the marked strains remains in translocated plants, evidenced by the isolation of the marked strain from *Trichoplusia ni* larval cadavers. Bt is vertically transmitted to the next generation of translocated *A. thaliana* via their seeds, keeping their insecticidal activity, tested by isolating the marked Bt strain from F1, F2 and F3 plants as well as from larval cadavers. Both marked Bt numbers and insecticidal activity increase throughout generations, indicating that Bt reproduce into the plant tissues. Sublethal effects are evident on surviving larvae. Interestingly, endophytic Bt strains are widely spread in nature. A total of 22 endophytic strains were isolated from the sap of 110 wild plants and 20 more from 72 wild plant seeds. Most isolates showed no significant identity to known Bt subspecies by flagellin and MLST sequences, except for some *nigeriensis* strains. The great majority show atypical parasporal crystals. From these isolates, only two showed moderate toxicity against *Manduca sexta* larvae and two more towards *Caenorhabditis elegans*. However, in a previous work, two strains isolated from lavender and Poinsettia sap showed significant toxicity when tested against *Aedes aegypti* and *M. sexta*, respectively. Their biotechnological significance is discussed.

CONTRIBUTED PAPERS **B-29**

Plants Recruit Insecticidal Bacteria to Defend against Herbivore Attacks

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Pest feeding affects the rhizobacteria community. The rhizomicrobiota activates salicylic acid and jasmonic acid signaling pathways to help plants deal with pest infestation. However, whether plants can recruit special pesticidal microorganisms to deal with attack from herbivores is unclear. A system composed of peanuts and first-instar larvae of *Holotrichia parallela* were used to analyze whether peanuts truly enrich the insecticidal bacteria after feeding by larvae, and whether inoculation of the enriched bacteria promotes the resistance of plants to herbivore. In this study, high-throughput sequencing of 16S rRNA gene amplicons was used to demonstrate that infestation of the subterranean pest *H. parallela* quickly changed the rhizosphere bacterial community structure within 24 hours, and the abundance of Enterobacteriaceae, especially *Enterobacter*, was manifestly enriched. Root feeding induced rhizobacteria to form a more complex co-occurrence network than the control. Rhizosphere bacteria were isolated, and 4 isolates with high toxicity against *H. parallela* larvae were obtained by random forest analysis. In a back-inoculation experiment using a split-root system, green fluorescent protein (*GFP*)-labeled *Enterobacter* sp. IPPBiotE33 was observed to be enriched in uneaten peanut roots. Additionally, supplementation with IPPBiotE33 alleviated the adverse effects of *H. parallela* on peanuts. Our findings indicated that herbivore infestation could induce plants to assemble bacteria with specific larvicidal activity to address threats.

CONTRIBUTED PAPERS **B-30**

Insights on *Pseudomonas protegens* ecology and bioinsecticidal properties

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Although the insecticidal activity of the *Pseudomonas fluorescens* group has long been known, the evolutionary specialization of its group member species *P. protegens* for insecticidal properties has only recently emerged. This ecological function is in addition to the abilities

of this gram-negative bacterium to interact with plants by inducing resistance and biostimulation. Studies on this multitalented species genome has revealed the existence of gene traits supporting the diversity of its biological activities. Our studies led to isolate a soil-dwelling strain stably interacting with soil nematodes. Bioassays have been conducted in the laboratory to evaluate its spectrum of action, which revealed its ability to cross intestinal barriers after oral administration followed by septicaemia and invasion of the haemocoel where it can exert its full pathogenic potential through the expression of specific virulence genes such as proteases, chitinases, and the FitD insecticidal protein. A broad spectrum of action against insects in different orders, including species of either agricultural and medical-veterinary interest (i.e., flies and mosquitoes) has also emerged. However, good selectivity to non-target species such as chrysopid predators was also observed, outlining a promising ecotoxicological profile.

Rapid adaptation of *Bacillus thuringiensis* to alkaline environments via the L-lactate metabolism pathway regulated by CRP/FNR family regulator LtmR

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Pathogenic bacteria adapt to the complex environment of the host through a variety of strategies that allow the bacteria to survive, evade host defenses, and establish infection. *Bacillus thuringiensis* (*Bt*) is an entomopathogenic bacterium that belongs to the *Bacillus cereus* sensu lato group comprising several species that are pathogenic to humans and animals. During infection, *Bt* often encounter alkaline conditions in the insect midgut, and the adaptation to this alkaline environment is crucial for its survival and establishment of infection. In this study, we investigated the rapid adaptation mechanism of *Bt* to an alkaline environment. DNA microarray data revealed 739 genes that were downregulated and 662 genes that were upregulated in the condition of 28 mM NaOH for 10 min relative to the condition without alkaline treatment. The activities of some primary metabolic pathways of *Bt* were enhanced under alkaline conditions, and many genes involved in the synthesis and transportation of amino acid, nucleic acid and the cell surface were significantly induced. Specially, the gene *ldh2* (HD73_5189) encoding a lactate dehydrogenase 2, and *lpm1* (HD73_0686) encoding a lactate-permease were significantly induced. The transcriptions of *ldh2* and *lpm1* were directly regulated by CRP/FNR family transcriptional regulator, LtmR (L-lactate transport and metabolism regulator), through the binding between LtmR and their promoters. The intercellular concentration of pyruvate was increased, and lactate was decreased under alkaline condition. Deletion of *ldh2*, the concentration of pyruvate was decreased, and lactate was increased, suggesting that *ldh2* is catalyzing the conversion of lactate to pyruvate. *ltmR*, *ldh2*, *lpm1* contributes to *Bt* virulence in *Ostrinia furnacalis*. Altogether, these data indicate that the pyruvate-L-lactate metabolic pathway is important for *Bt* adaptation and virulence under alkaline environment. It also provides new insight into the pathogen adaptation to host environments.

Exploring the Pathobiome of White Faeces Syndrome in *Penaeus vannamei*

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White faeces syndrome (WFS) is a syndromic disease affecting penaeid shrimp, specifically *Penaeus monodon* and *P. vannamei*. The syndrome manifests as white guts, retarded growth, high size variation, and loose exoskeletons in shrimp, with white faecal strings evident on the surface of the culture pond. WFS typically occurs 50-60 days post-stocking and can reduce yield by up to 60%, posing a significant threat to the shrimp industry. The aims of this study were to investigate the diseases associated with the WFS phenotype in *P. vannamei* and to explore the pathobiome contributing to WFS through 16S and metatranscriptomic sequencing.
We employed a combination of histological, metatranscriptomic, and bacterial composition analyses in shrimp affected by WFS. PCR screens were carried out to identify the presence of a selection of known pathogens, and 16S metabarcoding and metatranscriptomics (RNA-Seq) were used to characterise the microbial diversity in the samples. The PCR screens identified the presence of the microsporidian parasite *Enterocytozoon hepatopenaei* (EHP) in all shrimp with heavier infections observed in WFS-affected shrimp. No other known pathogens were detected by PCR. RNA-Seq identified the presence of a picornavirus, related to Wenzhou shrimp virus 8 (WZV8), which was more prevalent in WFS shrimp. Bacterial analysis revealed a depleted diversity in the hepatopancreas of shrimp with WFS, with a significant increase in *Vibrio* bacteria. Histological analysis showed more pathology in WFS-affected shrimp, most noticeable in the hepatopancreas, notably EHP spores, the infiltration of bacteria, and inclusions thought to be WZV8. Large quantities of necrosis were also observed.
Our work suggests that EHP, in combination with other pathogens such as *Vibrio* sp., and genetic factors, contributes to WFS. The presence of a picornavirus related to WZV8 was also identified, which was more prevalent in WFS shrimp. Future work is required to characterise the species of picornaviruses present in the metatranscriptomics dataset and determine if the bacterial infiltration seen in the hepatopancreas is *Vibrio*. This research adds to the current understanding of the complex aetiology of WFS and lays the groundwork for future studies.

Primed for success: Can immune priming be utilised in the Pacific oyster to improve resistance against *Vibrio aestuarianus*?

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Pacific oyster (*Crassostrea gigas*) production has faced widespread mass-mortality events in recent years, and the most notable pathogens of concern include ostreid herpesvirus 1 and the bacterium *Vibrio aestuarianus*. This project aims to investigate whether exposure to inactivated *V. aestuarianus* during early life stages of *C. gigas* primes an immune response to provide resistance upon re-exposure later in life,

and aims to investigate the molecular mechanisms underpinning this process.
We primed Pacific oyster veliger larvae and young spat from three biparental families with heat-inactivated *V. aestuarianus*, via bath exposure. Spat were challenged at 6-months-old via bath exposure of a fully virulent *V. aestuarianus*. Survival was monitored for 2 weeks and samples were taken for molecular analysis. We conducted transcriptomic analysis using RNA-Seq on whole spat samples collected 72 hours after challenge initiation to identify any alterations in gene expression between the primed and non-primed treatments.
Survival to 2 weeks following challenge initiation did not differ significantly between the primed and non-primed treatments. However, analysis of RNA-Seq data has revealed clear differences in gene expression between biparental families and an immune response in naïve oysters following *V. aestuarianus* challenge. We have found long-lasting alterations in the transcriptional response to infection associated with priming at both larval and spat stages. We now plan to conduct further work to investigate the hypotheses that differences in transcriptional responses associated with priming arise via epigenetic mechanisms and through interaction with surrounding microbiota. We expect the results to contribute to our understanding of the mechanisms via which priming of invertebrate immunity occur and how this can be utilised for improving disease resistance in oyster aquaculture.

Novel cell types and functions of *Crassostrea gigas* immune cells revealed by a comprehensive single cell transcriptomics and cytology atlas.

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Molluscs are one of the most diverse phyla among animals and are widely distributed worldwide in all types of environments, representing an important part of known animal biodiversity. However, their cellular physiology and function remain poorly described. The oyster *Crassostrea gigas* is a sessile bivalve widely distributed in natural beds and cultivated areas. In recent decades, *C. gigas* has suffered from recurrent microbial diseases with severe economic impact. Although increasing genomic data have revealed the diversification of its immune gene repertoires, the cellular and functional characterization of its immune cells, the hemocytes, remains poorly studied, despite being key components of oyster immunity. Limited knowledge of their diversity and functional properties has hindered a thorough understanding of critical host-pathogen interactions. Here, we investigated the diversity of hemocytes in *C. gigas* through in-depth integrative studies at the single-cell level. By combining scRNA sequencing with quantitative cytology, cell sorting and functional assays, we uncovered eight distinct transcriptomic and morphological circulating immune cell types. Beyond the classical three main cell types, blasts, hyalinocytes and granulocytes, we characterized four distinct granular cell types and potentially three distinct blast cells in addition to the hyalinocytes. Among the granular cells, only two cell types are capable of phagocytosis and only one has strong oxidative burst activity. Molecular markers specific for each transcriptomic cluster were identified, leading to an improved classification of *C. gigas* hemocytes. Finally, pseudo-time-ordering approaches allow us to propose a revised immune cell ontology. This study reveals the diversity of immune cells in *C. gigas*, highlighting the need to study this diversity in other molluscs and paving the way for the development of solutions to mitigate the impact of disease.

Growth of multiple strains of *Wolbachia* in the *Apis mellifera* cell line AME-711

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Honey bees (*Apis mellifera*) play a crucial role as pollinators of many different food crops worldwide. As such, research into the viral, bacterial and fungal pathogens that afflict honey bees, as well as into their ectoparasites such as the *Varroa* mite, is vitally important for understanding and alleviating these constraints on bee health. The world's only honey bee cell line AME-711, derived from *A. mellifera* embryos over a decade ago and persistently infected with the pathogen deformed wing virus (DWV), provides a convenient laboratory model system for investigating interactions between bee cells, bee viruses and intracellular bacteria such as the ubiquitous arthropod and nematode symbiont *Wolbachia*. Depending on the host species and the *Wolbachia* strain, these bacteria can influence replication of co-infecting viruses (such as dengue virus) or protozoa (such as the malaria parasite) either positively or negatively. As a first step towards investigating whether *Wolbachia* could be used as a tool to support honey bee health by suppressing or preventing infections with viruses such as DWV, we screened a panel of seven *Wolbachia* strains of differing insect origin for ability to replicate in the bee cell line. AME-711 cells were inoculated with cell-free bacteria derived from other persistently-infected insect cell lines and monitored by microscopic examination of live cells and Giemsa-stained cytocentrifuge smears for up to 6 weeks. Infections were established with 6/7 *Wolbachia* strains, usually within two weeks of inoculation. Strains *w*Pap and *w*CfeJ were maintained through more than five passages onto fresh AME-711 cells for up to one year; however, attempts to split already-infected cells were unsuccessful. The bee cell-*Wolbachia* culture system reported here can now be used to investigate and quantify the effect of bacterial infection both on the co-infecting DWV and on other bee pathogens introduced into the system.

The first isolation of a lactic acid bacterium (*Apilactobacillus kunkeei*) from honey bee (*Apis mellifera anatoliaca*) honey stomach and identification of its potential probiotic feature

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The Anatolian honey bee (*Apis mellifera anatoliaca*) is an important species in Türkiye, attracting attention with its unique characteristics and contributions to the environment. *Apis mellifera anatoliaca* is particularly valuable for Türkiye's biodiversity and beekeeping heritage. In this context, protecting the health of *Apis mellifera anatoliaca* is particularly important. Lactic acid bacteria (LAB) are very important for the health of bees. Probiotics are live bacteria that provide health benefits to the host when provided in appropriate amounts. This study aimed to identify and evaluate the antimicrobial and probiotic properties of a *Lactobacillus* bacteria isolated from *Apis mellifera anatoliaca*. As a result of morphological (Gram staining), physiological (catalase test), and molecular analysis (16s rRNA) studies, the bacterium was determined to be *Apilactobacillus kunkeei*. The 16S rRNA sequence was deposited in GenBank and accession number was obtained. The shape and arrangement of the bacteria were observed by scanning electron

microscopy (SEM) analyses. Probiotic properties were determined by the resistance to acid, pepsin, pancreatin, and bile salts.
Results showed that bacteria demonstrated good survival for up to 24 hours at all pH values tested. It showed antimicrobial activity against *Proteus vulgaris* ATCC13315 strain. Antibiotic susceptibility tests showed that the bacteria were susceptible to spectinomycin, nystatin, cycloheximide, kanamycin, ciprofloxacin, and streptomycin but resistant to erythromycin, ceftazidime, rifampicin, chloramphenicol, ampicillin, tetracycline, penicillin and gentamicin (10 mg/ml) antibiotics. All these results suggest that *Apilactobacillus kunkeei* has potential to be used as a probiotic.
Keywords: *Apis mellifera anatoliaca*, *Apilactobacillus kunkeei*, Microbiology, Lactic acid bacterium, Probiotic

CONTRIBUTED PAPERS **DBI-6**

CBPV exploits host AMPs to alter gut microbiota composition for viral infection

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Chronic bee paralysis virus (CBPV) exploits host immune to disturb microbiota for its proliferation remains elusive. Through histopathological examination, we discovered that the hindgut harbored the highest level of CBPV, and displayed visible signs of damages. The metagenomic analysis showed that a notable reduction in the levels of *Snodgrassella alvi* and *Lactobacillus apis*, and a significant increase in the abundance of the opportunistic pathogens such as *Enterobacter hormaechei* and *Enterobacter cloacae* following CBPV infection. Subsequent co-inoculation experiments showed that these opportunistic pathogens facilitated the CBPV proliferation, leading to accelerated mortality in bees and exacerbation of bloated abdomen symptoms after CBPV infection. The expression level of antimicrobial peptide (AMP) was found to be significantly up-regulated by over 1000 times in response to CBPV infection. In particular, through correlation analysis and a bacteriostatic test revealed that the AMPs did not exhibit any inhibitory effect against the two opportunistic pathogens. However, they did demonstrate inhibitory activity against *S. alvi* and *L. apis*. Our findings provide different evidence that CBPV utilizes the host's AMPs to eradicate probiotic species and facilitates the proliferation of opportunistic bacteria. This process weakens the intestinal barrier and ultimately resulting in the typical bloated abdomen.

CONTRIBUTED PAPERS **DBI-7**

Occurrence of honey bee pathogens in Vespa orientalis

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Vespa orientalis is spreading across Europe threatening wellbeing of honey bees by feeding on adult individuals and larvae and by plundering hive resources. The interaction between the two species could lead to possible spillover of pathogens. In the context of the EVOC project founded by Campania region, we investigated the possible presence of honey bee pathogens in adults and larvae of V. orientalis, namely Acute Bee Paralysis Virus (ABPV), Black Queen Cell Virus (BQCV), Chronic Bee Paralysis Virus (CBPV), Deformed Wing Virus (DWV), Kashmir Bee Virus (KBV), Sac Brood Virus (SBV), Nosema spp., Ascosphaera apis, Lotmaria passim, Chritidia spp. Adult hornets were collected from apiaries across southern Italy, while larvae were collected from a managed nest. Samples were subjected to anatomopathological analysis, followed by copromicroscopic, histopathological and biomolecular analysis. No morphological alterations were identified despite the biomolecular results showed 25/30 adults and 24/29 larvae were infected with at least one virus (DWV). Adult samples presented also ABPV (63%), BQCV (43%), SBV (3%,), KBV (3%); while larvae presented SBV (34%), ABPV (17%), BQCV (17%). No sample was positive for CBPV. Regarding parasites, copromicroscopic analysis revealed only the presence of Nosema spores in 38% samples. Histopathological analysis showed L. passim-like elements in the rectum of one examinate hornet and the presence of fungal hyphee in another hornet. Biomolecular analysis showed that N. ceranae was the most prevalent parasite (50%), followed by A. apis and L. passim (7%) and C. bombi (6%). All investigated individuals were negative for C. mellificae. Our results show that V. orientalis can harbor different species of honey bee pathogens despite not showing macroscopical alterations, therefore they could be silently contributing to their diffusion in the environment.

CONTRIBUTED PAPERS **DBI-8**

Enterococcaceae facilitates the proliferation of CSBV in honeybee by affecting metabolic disorder

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Chinese sacbrood virus (CSBV) is a serious threat to Asian honey bee, *Apis cerana*, especially larvae. Increasing evidence shows that the gut microbiome greatly influences viral infection. In this study, metagenome sequencing analysis showed that *Enterococcaceae* in silk group was significantly enriched, which mainly resulted in the formation of purulent cystic. Likewise, *Enterococcaceae* was main in honeybee larvae, not the adult. Subsequent co-inoculation experiments showed that *Enterococcaceae* facilitated the CSBV proliferation, leading to accelerated mortality in bees and exacerbation of gut damage after CSBV infection. In addition, *Enterococcaceae* treatment could affect metabolic disorder of honeybee for viral infection. Our findings illustrated and discussed the direct and indirect interaction between the virus-host and bacteria, this may provide a different evidence for intestinal symbiotic bacteria *Enterococcaceae* facilitating the proliferation of CSBV in honeybee larvae and resulting in the typical sac.

CONTRIBUTED PAPERS **DBI-9-STU**

In vitro cultivation of tsetse fly *Glossina fuscipes fuscipes* endosymbiont *Spiroplasma:* Genome sequencing and potential implications for disease transmission

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Tsetse flies (*Glossina* spp*)* are the cyclical vectors of the unicellular parasites African *Trypanosoma*, which are the causative agents of Human African Trypanosomosis (HAT), commonly known as sleeping sickness in humans, and African Animal Trypanosomosis (AAT) or Nagana in animals. These insects are of significant medical and economic importance and are among the major constraints for the socio-economic development of the African continent. In addition to their role in *Trypanosoma* transmission, tsetse flies harbour a diverse array of endosymbiotic bacteria, ranging from obligate mutualists to reproductive parasites. Among these, *Spiroplasma*, a helical, wall-less bacterium recently identified in *Glossina fuscipes fuscipes* (*Gff)*, has attracted attention due to its adverse impacts on host reproductive homeostasis. However, further evidence also suggests the potential role in modulating

vector competence by inhibiting *Trypanosoma* in laboratory settings. Recently, an *in vitro* bacterial culture of *Spiroplasma* from *Gff* was established, paving the way for further studies on the interactions with both its tsetse host and *Trypanosoma* at genomic and transcriptomic levels. The complete genome sequence of cultured *Spiroplasma* revealed a circular genome of 1489162 bp coding for 1868 genes. The findings may provide additional insights into the symbiotic relationship, revealing potential implications for vector competence and disease transmission.

CONTRIBUTED PAPERS **DBI-10-STU**

Iflavirus and Negevirus dynamics in mass-reared tsetse flies

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Mass rearing of insects is gaining in importance. One substantial niche of insect mass rearing involves the sterile insect technique (SIT) application. SIT is an insect control technique that needs healthy and competitive mass-reared insects. Given the high volume and high density of insects in mass-rearing facilities, pathogen transmission is fast but may stay undetected over long periods of time as in the case of covert viruses. Different abiotic and biotic factors can trigger a switch from covert to overt infection, which may lead to the collapse of the entire insect colony. How these virus infections are maintained or cleared remains poorly understood. In the tsetse fly rearing facility in Seibersdorf, Austria, Glossina morsitans morsitans iflavirus (GmmIV) and Glossina morsitans morsitans negevirus (GmmNegeV) have been monitored in different tsetse species. We observed that *Glossina pallidipes,* initially iflavirus and negevirus free, became infected with a new negevirus strain after exposure to infected tsetse colonies (*Glossina morsitans centralis*). In this project, the goal is to understand the dynamics of horizontal transmission and to characterize tsetse infecting negevirus strains in more detail. Therefore, we aim to obtain virus-free flies through a combination of clean feeding and the usage of anti-viral drugs. After clean feeding initially negevirus-positive *Glossina pallidipes* for 10 generations, the number of negevirus-positive flies was reduced (from 90% prevalence to 30%). We are now treating the infected flies with the antivirals ebselene and rupintrivir, believed to block viral 3C proteases. Furthermore, we amplified negevirus strains originating from different tsetse species (*Glossina morsitans morsitans, Glossina submorsitans, Glossina morsitans centralis, Glossina pallidipes*) via RT-PCR. We will sequence these amplified negevirus strains for further viral characterization.

CONTRIBUTED PAPERS **DBI-11-STU**

Reinfection and genetic diversity of Tenebrio molitor densovirus (TmDV) from Europe

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Yellow mealworms (*Tenebrio molitor*) have emerged as a significant alternative protein source in the feed and food industry over the past decade. However, little is known about viruses infecting this economically important protein source. Building upon our prior research, we have demonstrated the reinfection of a newly discovered densovirus in yellow mealworm in Europe, originally reported from metagenomics study of bird, known as Parus major densovirus (PmDV). The potentially novel virus, proposed to be named Tenebrio molitor densovirus (TmDV), was purified and subjected to ultracentrifugation before being introduced by feeding to a virus-free *T. molitor* population. Mortality was only observed in insects that were fed with very high doses of virus particles. Electron microscopy revealed that virus particles were approximately 25 nm in size. The virus was re-isolated from deceased samples and sequenced using Nanopore sequencing to ensure the integrity of the genome sequence. Interestingly, phylogenetic analyses revealed that the TmDV from Europe is more similar to PmDV, in contrast to TmDV isolated from the USA, where the virus only isolated from diseased sample. In order to analyse the intra-species genetic diversity of TmDV, single nucleotide variant (SNV) position were called among the samples from different life stages, using PmDV as reference. Variant analysis indicates the presence of a pure TmDV genotype in the larvae and pupae life stages, whereas mixtures of genotypes were observed in the adult life stage and healthy individuals of *T. molitor*. Our findings underscore the importance of understanding the infection patterns of TmDV within the yellow mealworm colonies worldwide.

CONTRIBUTED PAPERS **DBI-12**

Applying Ecotoxicology Principles in Mass-Reared Insects to Understand Stressor Interactions.

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Insect mass rearing is a rapidly expanding industry for production of food and feed protein. Insects reared in high density artificial rearing environments can be exposed to a range of biotic and abiotic stressors, including insect pathogens, potentially reducing growth rate, reproduction or inducing a population crash. Interactions between insect pathogens and other stressors can exacerbate effects of individual pathogens, yet understanding these interactions and reliably predicting combined stressor effects based on mechanisms of action is limited in insect pathology. To address this challenge, we reviewed how ecotoxicological modelling of multiple stressors can be applied to mass reared insect systems, adapting mixture theory concepts. This understanding is crucial for optimising rearing conditions for mass production, but can we extend these ecotoxicological principles into the field of insect pathology? Here, we propose a standardised methodology to enhance transparency across multiple stressor research fields, drawing on examples from ecotoxicology, microbial biological pest control and yellow mealworm (*Tenebrio molitor*) rearing. We discuss important considerations in multiple stressor terminology, experimental design, endpoints and analysis of results to advance understanding of multiple stressors and their impact on insects for food and feed.

The Tick Cell Biobank – cell lines for research on biology and control of insects, ticks, and their associated microorganisms

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Cell lines derived from arthropod vectors have played important roles in research on diseases of medical, veterinary, and agricultural importance for over 75 years. The need for new cell lines continues today as novel pathogens emerge and vectors invade new areas because of climate change and globalisation. The Tick Cell Biobank (TCB), the world's only dedicated culture collection for cell lines derived from ticks and other arthropods, specialises in generation of cell lines from neglected and challenging vector species. As well as housing and distributing a growing collection of tick and insect cell lines (alongside training in their maintenance) to scientists worldwide, the TCB is currently working on the generation of new cell lines from various species of ixodid and argasid ticks, mosquitoes, sand flies, triatomine bugs, fruit flies, honey bees, butterflies and ladybirds. The TCB also houses a small collection of intracellular, arthropod-borne bacteria including multiple strains of *Wolbachia*. Moreover, TCB Outposts in Malaysia, Kenya and Brazil facilitate cell line distribution in lower and middle-income countries. The TCB welcomes collaborations with scientists seeking to utilise cell lines in their research, who may lack expertise in this area or require assistance in sourcing or generating *in vitro* tools for their particular field of interest. Cell lines are distributed subject to Material Transfer Agreements; for further information, contact us at tickcellbiobankenquiries@liverpool.ac.uk.

Near-complete bacterial genome insertion in a tick nuclear genome: evidence from tick cell lines and ticks

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While rarely observed in eukaryotic animals, horizontal gene transfers (HGT) from prokaryotes into arthropod hosts are increasingly being reported. In earlier screening for bacterial endosymbionts, HGT of bacterial genes into the tick genome was initially suspected when cell lines generated from the tropical bont tick *Amblyomma variegatum* yielded positive PCR amplification of genes from the pathogen *Rickettsia africae*, known to be transmitted by this tick, even though visual examination of the cells did not show any signs of bacterial infection. Here we describe the investigations leading to discovery of an insertion of the nearly complete *R. africae* genome into the *A. variegatum* nuclear genome.
Tetracycline treatment of *A. variegatum* cell lines AVL/CTVM13 and AVL/CTVM17 did not deplete the *Rickettsia*-specific *gltA* gene when examined by quantitative PCR (qPCR), suggesting absence of actual bacteria in the cell lines. Proteomic analysis of both cell lines did not detect expression of any *Rickettsia*-specific proteins. Next generation sequencing (NGS) of AVL/CTVM17 cells provided evidence for the insertion of a nearly complete *R. africae* chromosome (~1.2 Mb) into the tick genome, although a ~54 kb region containing genes essential for metabolism was absent. Additional NGS data were obtained for *A. variegatum* ticks from a *Rickettsia*-free colony and the field, which were all *gltA* qPCR-positive. Both data sets showed the presence of nearly complete *R. africae* chromosome sequences and absence of the ~54 kb genomic region, corroborating the findings from AVL/CTVM17 cells.
In summary, we present multiple lines of evidence for bacterial genome insertion into the nuclear genome of the host tick. These findings represent the first such observation in ticks and highlight the potential impact of bacterial genome insertion on pathogen or endosymbiont surveillance in arthropods. We also demonstrate the value of arthropod cell lines in facilitating research on HGT in arthropod genomes.

Monitoring of larval growth and persistence of *Bacillus thuringiensis* and *Clostridioides difficile* spores during bio-conversion of waste streams by black soldier fly larva (*Hermetia illucens*)

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Among beneficial invertebrates, insects like the Black soldier fly, *Hermetia illucens,* larva (BSFL) are increasingly being considered as a sustainable animal feed ingredient. BSFL can grow on a variety of naturally microbe-rich substrates, like waste streams and agricultural by-products. BSFL are also known for their capacity to eliminate some pathogens, particularly through the production of antimicrobial peptides. However, little is known about BSFL ability to eliminate spore forming bacteria, that can survive routine hygiene treatments. In this study, we first evaluated the nutritional value and natural presence of spores from *Bacillus cereus* (Bc) group bacteria and *Clostridium difficile* (Cd). In five waste streams, three derived from plant material and two containing animal proteins Next, to assess the capacity of BSFL to reduce the spore load, the five waste (70% waste+30% wheat bran) were spiked with approximately 10^6 spores/g of *Bacillus thuringiensis* (Bt) or (Cd). 5 mg larvae were reared on the different waste streams (1 larva /gram feed), and larval weight and spore concentration were recorded, after 3 and 7 days, in the larvae and in the frass (mix of feed and faeces). All waste allowed a good larval growth; larval weight was ≈ 35 mg at day 3 and up to ≈180 mg at Day 7 for an animal protein waste. Some of the waste naturally contained *B. cereus* (≈10^4 cfu/g) or *Clostridium butyricum,* but no detectable Cd. The spiked Bt spores were recovered mainly as spores, in both the larvae and frass, without any significant difference in their final concentration according to the waste. However, a small, but non-significant difference, in the total spore counts, was found at day 3 or 7 as compared to day 0. Spiked Cd spores were not significantly reduced. In conclusion BSFL could not reduce Bt or Cd spores during bioconversion,thus the presence of spore-forming pathogens must be monitored in both larval diet and in the final larva to ensure safety.

Strongwellsea, a specialist genus of insect pathogenic fungi, shows remarkable species diversity

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Species in the insect pathogenic fungus genus *Strongwellsea* are specialists infecting adults from higher dipterans (Muscidae, Fanniidae, Anthomyiidae, Sarcophagidae, Scatophagidae and Calliphoridae). Upon infection, the host fly develops one, two or even three abdominal holes, through which primary conidia are actively discharged. These fungi may, instead of developing a hole and discharge conidia develop

thick-walled resting spores with brightly colored spiny epispores. During an intense sampling program in Denmark 2019-2023, we collected and described several new species from this genus, raising the number of recognized species from three to eight. They differ with respect to host species, conidial morphology and DNA. Additional new, still undescribed species are present in our material. We hypothesize that the genus contains a high number of mostly unknown species, infecting just one host species, excepting in some cases probably a few taxonomically closely related host species.

CONTRIBUTED PAPERS **F-2**

Diversity and impact of fungal pathogens infecting the spotted lanternfly, *Lycorma delicatula*

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Since 2014, the invasive planthopper *Lycorma delicatula* has been spreading in the eastern US, where it is of great concern as a crop pest and nuisance. In 2018 during a rainy period, we discovered massive mortality of dense populations during epizootics caused by *Batkoa major* (Entomophthorales) and *Beauveria bassiana* (Hypocreales). Sampling since that time, we have detected 17 additional species of fungal pathogens infecting *L. delicatula*. Since 2018, the most abundant of these pathogens has been *B. bassiana*, which has broad distribution with isolates from *L. delicatula* being genetically diversity. *Batkoa major* has a broad host range but infections declined over the few years after the 2018 epizootics and this pathogen was not detected during the dry summer of 2022. In recent years, diverse hypocrealean species were also identified infecting adult lanternflies, especially during the period in autumn when reproduction occurs. Fungal pathogens infecting spotted lanternflies in the US are naturally occurring generalists attacking this abundant new invasive host.

CONTRIBUTED PAPERS **F-3-STU**

The entomopathogenic genus *Beauveria* represents the predominant fungal pathogen among the adult *Popillia japonica* population in Europe

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Popillia japonica is an invasive and polyphagous beetle that causes major damages particularly in vineyards in the recently infested regions in northern Italy and southern Switzerland. Scientific studies have indicated that entomopathogenic fungi like *Beauveria* and *Metarhizium* are a promising option to control adult *P. japonica*. Development of successful biocontrol agents for aboveground application requires strains that are well adapted to prevailing environmental conditions such as temperature, UV radiation or humidity.
The aim of this study is to isolate well-adapted, predominant endemic fungal pathogens from *P. japonica* in the infested regions in Europe, with focus on *Beauveria* spp. and *Metarhizium spp*. We collected 200 adult beetles each in 17 vineyards across the infested area. The selection of vineyards included sites reflecting temporal advancement of *P. japonica* infestation (2015 to 2022) to determine whether the duration of *P. japonica* presence had an impact on pathogen community composition. Beetles were monitored for fungal infections. Most of them died and mycosed between two and four weeks after collection. Based on morphology, 615 isolates of mycosed individuals were assigned to *Beauveria* (566) or *Metarhizium* (49). The number of isolates per vineyard was highly variable and initial analyses revealed no correlation between fungal abundance and duration of *P. japonica* presence, suggesting other factors like geographic origin, climatic conditions, and farming practice (organic or conventional) or other hosts may drive fungal abundance. Next, marker loci (Bloc or TEF1) will be sequenced and microsatellite marker analyses performed to allow species and genotype assignment of all isolates. Genetic diversity will then be correlated to factors mentioned above. This study will provide an important base to identify potential biocontrol strains that are virulent against *P. japonica* and optimally adapted to environmental factors in the infested regions.

CONTRIBUTED PAPERS **F-4**

Exploration of intraspecific lineages and genetic diversity of *Beauveria pseudobassiana*

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Since its first description in 2011, the entomopathogenic fungus *Beauveria pseudobassiana* has been reported as a pathogen of multiple insect species. Recently, *B. pseudobassiana* has also been reported as the main pathogen of *Melolontha melolontha* adults, an insect pest in Europe. Despite increasing interest, the intraspecific phylogenetic composition and ecological niches occupied by *B. pseudobassiana* remain poorly investigated. To determine its distribution and genetic structure within *M. melolontha* habitat, we aimed to introduce novel phylogenetic and population genetic frameworks for *B. pseudobassiana*. Therefore, 37 *B. pseudobassiana* isolates were collected from diverse sources (i.e., *Melolontha melolontha* adults, soil, grassland plants and tree leaves) at two sites in Switzerland. A phylogenetic analysis of the nuclear marker Bloc included the 37 collected isolates and a non-redundant reference set of 78 *B. pseudobassiana* GenBank sequences and 21 genome accessions, representing isolates originating from diverse geographic locations and insect hosts. Sequences of ATPase 1 (MDN1) from the reference genomes were also included to improve resolution and branch support. Concurrently, we developed 18 microsatellite markers and applied them to the 37 Swiss isolates to explore population genetic structures. Preliminary results revealed congruent genetic clustering of the Swiss isolates between Bloc-sequence and microsatellite analyses suggesting that *B. pseudobassiana* comprises a cryptic species complex. The 37 *B. pseudobassiana* isolates were distributed among four divergent clusters, with no observed correlation with either geographic origin or isolation source. Microsatellite markers will facilitate future exploration of the distribution and ecological niche associations of *B. pseudobassiana*, and the phylogenetic framework a pathway to a robust intraspecific phylogeny and delimitation of cryptic species boundaries.

CONTRIBUTED PAPERS **F-5-STU**

Microbiome of North American Ash for Biocontrol of Emerald Ash Borer

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Ash (*Fraxinus*) are economically and culturally important trees in North America, and host to numerous native wood boring beetles and their parasitoids. However, little is known about their microbiota and, more specifically, endophytic or pathogenic fungi that may grow in the living phloem and leaves. The Emerald Ash Borer (EAB; *Agrilus planipennis*),

an invasive phloem feeding beetle from Asia, now threatens all native North American ash Species. We hypothesized 1) that increasing infestation with EAB will decrease endophyte diversity and abundance in ash phloem and 2) that ash trees may harbor endophytes that are entomopathogens of EAB. To investigate these questions, we collected infested and uninfested phloem, insect frass, and insects in two regions of New York. Cultured fungal isolates included several potential entomopathogens from families Cordycipitaceae, Ophiocordycipitaceae, and Clavicipitaceae, as well as other insect-associated fungi that may be plant pathogens (Ophiostomataceae) or saprophytic wood rot (Peniophoraceae) fungi. Several entomopathogenic taxa were isolated not only from insects, but also from galleries, frass, and even uninfested ash phloem, suggesting they may grow within healthy phloem as entomopathogenic endophytes. Our findings will elucidate the diverse fungi in this system, identifying fungi with potential roles in ash-decline, as well as entomopathogenic endophytes, which could offer novel options for biocontrol of this invasive insect.

CONTRIBUTED PAPERS **F-6**

Fungal parasites of nematode eggs for biocontrol of the soybean cyst nematode

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Fungal parasites of nematodes occupy unique niches in the environment and have evolved sophisticating mechanisms for sensing and parasitizing their prey. Nematode trapping fungi are best known because of their charismatic constricting rings that actively capture nematodes in soil, but two other ecological guilds of fungal nematode parasites, including those attacking nematode eggs (egg parasites) and those that parasitize the juvenile worms in soil (endoparasites), also have potential for biocontrol of nematode pests. Our work investigated fungal egg-parasites of the soybean cyst nematode (*Heterodera glycines*; SCN), a damaging pathogen of soybean in the U.S. and globally. Using culturing, metabarcode sequencing (16S V3V4 and ITS1 regions), and shotgun metagenomics of SCN cysts, soil, and soybean roots from a long-term agricultural field, we identified microbial pathogens enriched in soils suppressive to the soybean cyst nematode, including several early diverging lineages of fungi that have not previously been identified as nematode parasites. In-vitro bioassay testing identified fungi antagonistic to SCN, distinguishing those that are parasites directly infecting and killing live nematode eggs from those that may secrete bioactive secondary metabolites or enzymes toxic towards nematodes. Using genomic and transcriptomic approaches, we evaluate possible mechanisms of nematode antagonism among egg-parasites versus toxin producers. As many of these egg-parasitic fungi also colonize plant roots as endophytes and showed efficacy in preliminary greenhouse trials, they show promise either as seed or root bio-inoculants for integrated pest management of plant pathogenic nematodes in agriculture.

CONTRIBUTED PAPERS **F-7**

Production and formulation of nematopathogenic fungi for control of cyst nematodes

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Plant-parasitic nematodes like *Heterodera schachtii* cause economically relevant yield losses. Since synthetic nematicides have been withdrawn in Europe, nematode control is limited. A sustainable alternative for control of nematodes are nematopathogenic fungi.
Fungal strains JKI72728 (not identified), JKI72954 (*Pochonia chlamydosporia*), JKI72955 (*Pyrenochaeta* spec.), JKI72956 (*Exophiala* spec.), JKI72994 (not identified) and JKI73030 (*Niesslia* = *Monocillium gamsii*) were isolated from nematode eggs and provided by JKI-EP. We investigated characteristics, which may be important for further development of a biocontrol agent. Based on radial growth measurements we compared the temperature optima. For JKI72956 the optima were at 15-25 °C, for JKI72994 15-20 °C and for the other strains at 25 °C. JKI72728, JKI72954 and JKI7295 were growing faster than the other two.
Next, we looked for the most efficient production system. Within solid-state fermentation only JKI72954 produced countable numbers of spores. In liquid flask culture JKI72954 and JKI72956 were producing spores of up to 5×10^8 spores ml^{-1}. When JKI72954 was cultivated in a liquid fermenter the spore yield was increased to 2×10^9 spores ml^{-1}.
For formulation the liquid fermented spores were suspended in different sugar solutions and were freeze- or spray-dried. The germination rate was determined before and after drying. When JKI72954 was freeze-dried, germination rates of 76 % were obtained by using glucose followed by sucrose (66%) as cryo-protectant. For JKI72956 a germination rate of only 18 % was achieved. Using the same protectants the viability of JKI 72954 after spray-drying was 83 % (fructose) and 79 % (glucose), respectively. For JKI72956 a germination rate of 60 % (glucose) and 55 % (fructose) was achieved.
Based on these results it looks promising that these two strains can be produced and formulated. The next step is to proof the efficacy of formulated submerged spores against cyst nematodes.

CONTRIBUTED PAPERS **F-8**

Soil treatments with *Metarhizium brunneum* Petch. (Ascomycota: Hypocreales) for the control of the olive fly *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) can promote olive tree growth

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The research group AGR 163 Agricultural Entomolog has developed, for over 20 years, a control method for the olive fly *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) through soil treatments with entomopathogenic fungi (EF). The treatment with the strain EAMa 01/58-Su of *Metarhizium brunneum* Petch. (Ascomycota: Hypocreales) reduces the spring population by up to 70%. Additionally, the EF have shown beneficial side effects on the plants that recieve the treatment, by behaving as endophyte or competent microorganism in the rhizosphere. This work shows how the EAMa 01/58-Su strain behave as rhizosphere competent in the olive plants, and colonizes the plant at the root cortex level producing higher colonizations in the Picual variety than in the Manzanilla. The treatments were performed with the two propagules, conidia and microsclerotia, and the interaction with mycorrhiza was included. The colonization of the plant by the fungus has been detected both by microbiological techniques and by qPCR and ddPCR. The strain

has produced greater length of secondary branches and thicker stems in all treatments with conidia in the Picual variety. Finally, most of the treatments where microsclerotia were applied induced certain genes of the ethylene pathway in the Manzanilla variety. Regarding jasmonic acid, only the treatment with mycorrhiza and microsclerotia showed differences compared to the control in both varieties. No treatment from the salicylic acid pathway was significantly different from its control.

CONTRIBUTED PAPERS **F-9-STU**

Symbiotic bacterial abundance and protective response of two-spotted spider mites against *Akanthomyces attenuauts* JEF-147

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The two-spotted spider mite (*Tetranychus urticae* Koch) is an agriculturally serious polyphagous pest that has acquired strong resistance against acaricides because of its short life cycle and continuous exposure to acaricides. As an alternative, mite-pathogenic fungi with different modes of action could be used to control the mites. The spider mite has symbiotic microorganisms that could be involved in the physiological and ecological adaptations to biotic stresses. In this study, mite-pathogenic fungi were used to control female adults, and the microbiomes changes in the fungus-infected mites were analyzed. The acaricidal activity of 77 fungal isolates was tested, and *Akanthomyces atttenuatus* JEF-147 exhibited the highest acaricidal activity. Subsequently a dose -response assay and morphological characterization was undertaken. For microbiome analysis in female adults infected with *A. attenuatus* JEF-147, 16S rDNA and ITS1 were sequenced using Ilumina Miseq. Infected mite showed a higher Shannon index in bacterial diversity but lower index in fungal diversity. In beta diversity using principal component analysis, JEF-147-treated mites were significantly different from non-treated controls in both bacteria and fungi. Particularly in bacterial abundance, arthropod defense-related *Rickettsia* increased, but arthropod reproduction-associated *Wolbachia* decreased. The change in major bacterial abundance in the infected mites could be explained by a trade-off between reproduction and immunity against the early stage of fungal attack. In fungal abundance, *Akanthomyces* showed up as expected. Foremost, this work reports microbiome changes in a fungus-infected mite and suggests a possible trade-off in mites against fungal pathogens. Now RNA-sequencing of infected mites is under progress to elucidate the mite response against the fungal infection.

CONTRIBUTED PAPERS **F-10-STU**

Unraveling DNA methylation in the entomopathogenic fungus *Metarhizium pinghaense* NCHU-125

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Understanding epigenetic mechanisms of entomopathogenic fungi (EPF) is crucial for deciphering the different growth patterns, however, the regulation of methylation in EPF is still unclear. In this study, the whole genome of *Metarhizium pingshaense* NCHU-125 (Mp-NCHU-125) was sequenced using Oxford Nanopore Technologies (ONT) and next-generation sequencing (NGS). The genome size of Mp-NCHU-125 is 40 Mbp including 10 contigs, and the global methylation level of conidia (CG= 2.42%, CHG= 0.67%, CHH= 0.32%) is higher than those of mycelium (CG= 1.87%, CHG= 0.44%, CHH= 0.21%). Furthermore, different methylated regions of two fungal developmental stages were identified, revealing that methylation of conidia and mycelium primarily occurs at the transcription start and termination regions of genes, while methylation in transposable element (TE) regions is primarily located within the TE, suggesting that methylation might crucial for EPF developmental patterns. To investigate the influence of methylation, the Mp-NCHU-125 was treated with 5-Azacytidine (5-aza). Interestingly, after 10 days treatment, the conidia productivity was 2.88-fold higher than that of control, indicating the influence of methylation on EPF. Therefore, the methylomes of 5-aza treated or un-treated Mp-NCHU-125 were unveiled by ONT. The methylation levels of 5-aza treatment (CG= 2.2%, CHG= 0.63%, CHH= 0.3%) was significantly lower than control (CG= 3.02%, CHG= 0.76%, CHH= 0.4%).Through the comparative genomics of 5-aza treated or un-treated Mp-NCHU-125, the movement of 43 TE regions were identified. Besides, transcriptome analysis revealed 422 up-regulated and 834 down-regulated genes after 5-aza treatment. Among these regulated genes, six transposases were found, assumed the activation of TE might correlate to the demethylation in EPF. These findings indicate that methylation might affect the gene expression levels and TE activation. The validation of transposase expressions after 5-aza treatment will be discussed in the future.

CONTRIBUTED PAPERS **F-11**

"Unraveling multi-organismal interactions in the mosquito holobiont to enhance microbial control"

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The intricate molecular interactions between the invading entomopathogenic fungi, the mosquito and its microbiota are crucial for defining the outcome of the infection. Knowledge of these multipartite interactions can provide critical insights into mosquito biology and are important pillars in the design of microbial control strategies. In this regard, we have evaluated canonical pathogen recognition receptors and the antimicrobial effectors induced during the infection process. While pathogen recognition receptors are critical for microbial detection and initiation of the immune response, the antimicrobial effectors are paramount to the outcome of infection. Our studies evaluating the fungal infection process with distinct fungal entomopathogens indicates that fungal infection induces the expression of lysozyme, cecropin, diptericin, holotricin but do not affect those of attacin and gambicin. The elicitation of these antimicrobial effectors might play a role on the dysbiosis observed during mosquito infections with distinct fungal entomopathogens. RNAi-based depletion of select AMP transcripts indicates that these antimicrobial effectors are playing in concert rather than individually to potentiate their antifungal effect against fungal entomopathogens.

CONTRIBUTED PAPERS **F-12**

The body-surface associated microbiome of soil-dwelling larvae of *Melolontha melolontha* increases resistance to its fungal pathogen *Beauveria brongniartii*

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The microbiota living on insect body surfaces have been reported to affect susceptibility of insects like *Drosophila melanogaster* and *Delia antiqua* towards fungal infections. Axenic insects have been shown to be more susceptible and if re-inoculated with bacteria cultivated from insect surface have revealed delayed or reduced mortality.
The goal of the present study was to test whether the microbiome present on soil dwelling larvae of the cockchafer *Melolontha melolontha*

(*Scarabaeidae*), a pest feeding on roots of grassland- and crop plants, affects its susceptibility towards the fungal pathogen *Beauveria brongniartii*. *M. melolontha* larvae collected from a grassland field in Switzerland were dip-treated with an antibiotic/fungicide cocktail to reduce or remove the surface microbiome. Subsequently, treated larvae were washed with sterile water and dip-infected with a suspension of 10^7 conidia / ml of *B. brongniartii* strain BIPESCO4. Treatments and controls each included a total of 60 larvae (3 replicates of 20 larvae) and survival of the larvae was monitored for 45 days. All the antibiotic treated larvae died within the course of the experiment whereas 20% of the larvae that did not receive the antibiotic treatment survived. Overall median survival of the microbiome depleted larvae was significantly lower (14 d) as compared to the ones harboring their native microbiomes (23 d). Larval survival was not affected by the antibiotic treatment alone (5% dead after 45 d). Our results support the importance of the surface-microbiome for disease suppression on soil dwelling larvae like those of *M. melolontha*. Composition and function of the surface-microbiome of this insect in soils of different grassland or crop fields remains to be further elucidated.

CONTRIBUTED PAPERS **F-13**

Influence of defense mechanisms of *Euschistus heros* (Hemiptera: Pentatomidae) during the infective process of *Metarhizium* spp.

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It has been hypothesized that the low susceptibility of the soybean bug *Euschistus heros* to infection by entomopathogenic fungi has been associated with the emission of volatile compounds capable of inhibiting conidial germination. In this context, the study aimed to understand the interaction between entomopathogenic fungi and the volatiles released by *E. heros* using a virulent isolate of *M. pingshaense* (ESALQ 4395) and an isolate of *M. anisopliae* (ESALQ E9) that was not effective in controlling the species. *E. heros* were immersed in fungal suspension, kept at 26ºC for different periods, and photographed by SEM. The images indicated that germination starts approximately 18h after infection, and the presence of the metathoracic gland does not seem to influence this pattern. The emission of volatiles was evaluated with healthy and infected insects, and the compounds were identified by GC-MS and quantified by GC-FID. The emission did not differ between infected and healthy insects, and the major compounds were (E)-2-hexenal, (E)-2-octenal, 4-oxo-(E)-hexenal, and tridecane. Then, the isolates were exposed in two different ways to these compounds alone and in combination to evaluate the effect on fungal germination and growth. The lowest concentration of 10µg for all treatments did not negatively affect fungal germination and growth. However, the concentration of 10µg for the blend treatment under the ESALQ 4395 seems to stimulate fungal germination compared to the control. The results suggest that the documented negative effect only occurs at concentrations higher than the regular release observed in the extraction test. Furthermore, the concentration that mimics the natural release of these volatiles appears to have some beneficial interaction with *M. pingshaense*, which may be related to its greater virulence.
Keywords: Alarm pheromone; volatile compounds; microbial control.
Financial support: FAPESP, projeto n°2018/02317-5.

CONTRIBUTED PAPERS **F-14**

RNA-sequencing of entomopathogenic fungus-infected *Thrips plami* reveals change of host defense and homeostasis

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Entomopathogenic fungi could be effectively used to manage melon thrips populations with resistance against chemicals. In this work, a colony of melon thrips adults was infected by a fungal isolate and transcriptional response of infected thrips was investigated to figure out how the thrips responded during fungal pathogenesis. For Illumina sequencing, RNAs were extracted from the non-treated thrips and day-2 and day-4 infected thrips with three biological replicates. A big significant change of gene expression was not detected between non-treated control and day-2 thrips, but day-4 thrips showed many differently expressed genes. In the day-4 thrips enriched lysosome and insect hormone biosynthesis pathways were remarkably suppressed, although some other pathways were actively expressed such as serine and glycine metabolism, Toll/Imd and circadian rhythm pathways. Many lysosomal hydrolase genes including protease, glycosidase, sulfatase and lipase were significantly down-regulated and particularly glycosidases were strongly down-regulated. Some hydrolase precursor-related genes at Golgi body were actively expressed, but they didn't further proceed to the hydrolase biosynthesis. Juvenile hormone biosynthesis was up-regulated at the up-stream of the pathway, but many genes were significantly down-regulated at the down-stream, finally failing in juvenile hormone biosynthesis. In ecdysone biosynthesis, cytochrome P450 genes at the down-stream were up-regulated, but at the up-stream expressions of cholesterol desaturase and P450 gene were inhibited, consequently down-regulation of ecdysone biosynthesis. Compared to currently used chemicals mainly targeting neurotransmission and energy production, this fungus seems to attack different organs or pathways in the thrips. The addition of entomopathogenic fungus to the chemical spray calendar or tank-mixing with chemical insecticides would be a practical application method to manage the resistant thrips populations.

CONTRIBUTED PAPERS **F-15**

To be or not to be entomopathogenic depends on a mycovirus

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Fungal entomopathogenic ascomycetes (EA) are becoming a key biocontrol tool in Integrated Pest Management while increasing evidence highlight their role as multipurpose plant-beneficial microorganism for sustainable agriculture. The research on the virulence factors of EA is important from the perspective of accelerating their speed of kill or to reduce the fungal dosage for the required control levels. However, it could also be important in the opposite direction, to obtain non-pathogenic strains that could be used as beneficial plant symbiotic microorganisms with growth promotion abilities. There are several virulence factors described for EA with varying levels of importance, whereas hereby, it is shown for the first time that a mycovirus is an absolute virulence factor of a *Beauveria bassiana* (Balsamo) Vuil. strain, determining whether pathogenicity exists or not. The wild mycovirus-infected *B. bassiana* strain, which it is also an endophytic strain with demonstrated growth promotion abilities, is highly virulent against the experimental insect host *Galleria mellonella* L., whereas the cured strain that has lost the mycovirus is not pathogenic, with its cuticle penetrating activity suppressed. Moreover,

the wild mycovirus-infected strain is a highly Pr1 producer and exhibits elevated enzyme activity of extracellular (cuticle-degrading) enzymes (ECE) relevant to virulence, whereas in the mycovirus-free strain, the secretion potentials of both Pr1 and ECE are suppressed. These results are discussed in terms of how the mycovirus could be exploited to obtain hypervirulent EA strains but also potentially to transition an EA from entomopathogenic to solely plant-beneficial microorganism, which would likely require accommodation within the present legislative framework for the use of EA as plant-beneficial microorganisms.
Funding was provided by the Spanish Ministry of Science and Innovation via Grant PID2022-140233OB-I00.

CONTRIBUTED PAPERS **F-16**

Zinc solubilization and organic acid production by the entomopathogenic fungus, *Metarhizium pingshaense* sheds light on its key ecological role in the environment

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We report for the first-time higher zinc (Zn) solubilization efficiency and plant growth promotion by an entomopathogenic fungus (EPF), *Metarhizium pingshaense* IISR-EPF-14, which was earlier isolated from *Conogethes punctiferalis*, a pest of global importance. We observed that the Zn solubilizing efficiency of the fungus varied depending on the type of insoluble source of Zn (ZnO or $Zn_3(PO_4)_2$). We ascribe the production of various organic acids such as gluconic, keto-gluconic, oxalic, tartaric, malonic, succinic and formic acids as the major mechanism for Zn solubilization by *M. pingshaense*. Application of the fungus alone and in combination with insoluble Zn sources enhanced various plant growth parameters such as number of leaves, leaf length and width, shoot and root length, shoot and root dry weight and chlorophyll content in rice and cardamom plants. Moreover, the uptake of Zn in rice plants was also enhanced by fungal application. The fungus also exhibited various other direct and indirect plant growth-promoting traits, such as production of IAA, ammonia, siderophores, solubilization of mineral phosphate, and production of hydrolytic enzymes such as α-amylase, protease, and pectinase. Plant growth promotion by this fungus was observed to be multifarious and insect independent. Our findings shed light on the broader ecological role played by this fungus and widen its scope for utilization in sustainable agriculture.

CONTRIBUTED PAPERS **MC-1**

Opportunities and challenges for microbial control of arthropod pests on the US West Coast

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California, Oregon, and Washington are three states on the US West Coast where the value of various agricultural commodities is around $75 billion. High-value specialty crops such as small fruits, tree fruits, nut crops, and vegetables are among hundreds of commodities produced in these states that are vulnerable to numerous endemic and invasive pests. Although microbial control as a part of integrated pest management (IPM) has been advocated for decades, in general, the biopesticide market has grown only in recent years due to increased demand for non-synthetic pesticide options and the availability of several biopesticide formulations in response to this demand. The recent sustainable pest management roadmap in California emphasizes IPM practices and promotes the use of non-synthetic alternatives including biopesticides. However, conversations with growers, crop care professionals, and biopesticide industry representatives and anonymous surveys from various groups indicated several opportunities and challenges for microbial control. Lack of research and research funding, information on best management practices, higher cost of pest management with biopesticides, and concerns for control efficacy are among the major challenges. Policies that continue to restrict the use of synthetic pesticides, the untapped potential of microbial control in Oregon and Washington, and the limited use of biopesticides in California are among some opportunities to focus on microbial control research and outreach. This presentation will look at the current status of microbial control in California, Oregon, and Washington, input from the farming communities and biopesticide industry, and explore the next steps for promoting microbial control.

CONTRIBUTED PAPERS **MC-2**

Development, characterization, formulation, and use of bioinsecticides based on Baculovirus and *Bacillus thuringiensis* in Brazil.

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The use of biopesticides based on Baculovirus and *Bacillus thuringiensis* in Brazil has increased at a rate of approximately 30% per year. Embrapa Maize and Sorghum has a Multifunctional Microorganism Collection with 11,000 accessions and, 4,500 of which are *Bacillus thuringiensis* and 200 Baculovirus isolates. The projects focus on the isolation and molecular characterization of Bt genes for future transgenic plants and, the use of Bt and baculovirus for the development in small scale and formulation of biological products to control *Spodoptera frugiperda*, *Helicoverpa armigera*, *Chrisodeixys includens*, *Heliothis zea*, and other important corn, cotton, and soy pests. To date, Embrapa Maize and Sorghum has developed 11 (eleven) biological products that have been registered by private companies and placed on the market. These products have generated clones with other companies and are also on the market. The products developed based on baculovirus to control fall armyworm are: CartuchoVit®, BaculoMip®, Spodovir®, Vir Control Sf®, Destroyer®, Virumix®, for fall armyworm, and VirContrl Ci® for soybean looper, and for *H. armigera* Vir Control Ha®. Bt-based products for the control of fall armyworm and soybean looper are: Acera®, Crystal® (fall armyworm only) and Bt3®.

CONTRIBUTED PAPERS **MC-3**

Use of baculoviruses into conventional agriculture in the United States

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The use of baculoviruses in the United States is not new. In the 1970's, The United States Department of Agriculture Forest Service received Environmental Protection Agency (EPA) registration of baculoviruses to manage the forest pests *Orgyia psuedotsugata and Lymantria dispar.* A number of baculovirus products have received EPA registration in conventional agriculture since them, but few were commercially successful. Growers are now starting to view baculoviruses as a viable pest management tool for lepidopteran pests. Reasons for this includes an increase in insecticide resistance, few new modes of actions, high cost of newer products and competitive baculoviruses prices. Companies are collaborating with crop consultants and university research and extension entomologist to

properly educate growers on the use of baculoviruses to meet their need.

CONTRIBUTED PAPERS **MC-4-STU**

Surviving a baculovirus infection: impact on life history traits and potential resistance development in the fall armyworm *Spodoptera frugiperda*

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Baculoviruses are widely used for the biological control of several insect pests and have the potential to combat the invasive fall armyworm (FAW), *Spodoptera frugiperda*. When applied in the field, mortality is rarely 100% and a subset of larvae survive the infection. Our study focused on the impact of surviving a viral infection on FAW fitness and potential resistance development in this invasive pest. We infected second instar larvae during each of nine generations with a concentration of Spodoptera frugiperda multiple nucleopolyhedrovirus (SfMNPV), which was estimated to kill 50% of the population (LC_{50}) and recorded mortality and life history traits of surviving larvae. Additionally, at the F1, F4, and F8 generations, we infected a subset of both the control and infected groups with a lethal concentration of SfMNPV required to kill 80% of the population (LC_{80}). In the LC_{50} infected group, we found a reduction in mortality over the generations from ~46% in the F0 generation to ~20% in the F8 generation, while in the lethally infected population we found reductions of 75% to 60%, respectively. Furthermore, the continually infected group had a significantly longer developmental time – from larvae to pupae and pupae to adult, and a lower fecundity compared to the control group. The findings from our study provide valuable insights into the possible impacts of surviving virus infection on FAW fitness and resistance development.

CONTRIBUTED PAPERS **MC-5**

A novel Cydia pomonella granulovirus (CpGV) isolate overcomes type II resistance in codling moth

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Granulovirus application is a strong and selective tool for codling moth (CM) control in IPM and organic pome fruit production. Since first description of CM resistance towards virus isolates of genome group A used in commercial virus products in 2005, new candidates of resistance-breaking CpGV isolates have been subsequently substituted in products to overcome field resistance. Isolates from genome group B, such as CpGV-E2, are currently the only available resistance-breaking CpGVs to overcome both type I and II resistance in CM field populations. Here we describe a new virus isolate discovered from covert infection in our laboratory CM rearing. The 123.23 kb long CpGV-B, phylogenetically assigned to genome group E, shows 99.3% genome similarity to the reference genome CpGV-M (genome group A) and, *inter alia*, differs by a fusion of orf26 and orf27 with yet unclear effect. Infection bioassays of CpGV-B in the laboratory strain CpR5M expressing type II resistance with dominant, autosomal inheritance pattern showed higher mortality rates than infection of CpGV-E2, suggesting CpGV-B being an alternative resistance-breaking isolate useful for complementary application in operations with verified lowered CpGV-E2 efficacy. Transcriptomic analysis revealed differentially expressed host immune genes after inoculation with CpGV-B or CpGV-E2 in type II resistant laboratory CM strain (CpR5M). Additionally, both tested isolates differed in expression levels of certain viral infectivity genes after infection in the resistant strain. Further investigations on midgut-specific cellular infection responses and pathway will help understanding potential ability of CpGV-B to suppress or escape the host immune response.

CONTRIBUTED PAPERS **MC-6-STU**

Efficacy of a spray-dried formulation of Bacillus thuringiensis subsp. kurstaki (strain LIP) produced on a wheat bran-based complex medium.

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Biopesticides based on *Bacillus thuringiensis* (*Bt*) are a safer alternative to chemical pesticides in agriculture. Its insecticidal activity is due to the production of crystalline proteins known as Cry-toxins. Wheat bran (WB) is a low-cost industrial by-product that contains all the necessary nutrients for Bt growth and sporulation. Previously, in the context of the IPM-4-Citrus project, we successfully formulated a fluid bed-dried product. Our current European follow-up project SAFWA seeks to optimize bioproduction processes by cultivating *Bacillus thuringiensis* subsp. *kurstaki* (*Btk*) in a WB-based complex medium at Julius Kühn-Institute, Dossenheim. This work aims to develop an efficient method to formulate the active ingredient and to assess and improve its efficacy against two Lepidopteran insect pests: *Spodoptera frugiperda* and *Tuta absoluta*. The strain used was *Btk* strain "LIP" isolated from Lebanese soil. Fermentations were carried out using a five-litre fermenter (Minifors, Infors, CH). From a three-liter fermentation broth of LIP, 7 to 10 g dry weight of biomass was collected. Biomass without and with additives was spray-dried using a mini-spray dryer (S300, Buchi, CH) and bioassays were conducted against *S. frugiperda* to determine the lethal concentration (LC50). It is confirmed that the efficacy of spray-dried formulations was comparable to both fluid bed-dried formulations used in IPM-4-Citrus and reference commercial product DELFIN-WG. Based on the results of laboratory bioassays, the efficacy of the spray-dried unformulated and formulated biomass of LIP, along with the reference commercial product DELFIN-WG, will also be compared by realizing a field trial involving tomato plants against *T. absoluta* in Adana, in collaboration with Çukurova University. Field trial results will be discussed in terms of efficacy, UV resistance, and soil residual tests.

CONTRIBUTED PAPERS **MC-7-STU**

Endophyte *Beauveria bassiana* (Balsamo) Vuill. modifies cotton aphid responses: RNAseq analysis of *Aphis gossypii* Glover in response to feeding on endophytically colonized plants

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The cotton aphid has been shown to be susceptible to endophytic entomopathogenic ascomycetes (EEA), either in plant or soil sprays or seed dressing, when the aphid specimens feed on transiently or systemically colonized plant tissues, whereas the causes of the observed lethal and sublethal reproductive effects remains unknown. Indeed, it has been detected that factors other than the typical

integumentary mode of action of the fungal propagules colonizing the plant could be involved, with emphasis in the induction and modulation of the plant defence responses or plant-related stress-induced activation in the pest. In this study, we conducted transcriptome analyses of cotton aphids (Aphis gossypii Glover) and their symbiotic bacteria after feeding on plants colonized by the entomopathogenic fungus *Beauveria bassiana*. RNA-Seq expression profiling revealed dynamic changes in a total of 95 differentially expressed genes, 89 aphid genes and six *Buchnera aphidicola* Munson et al., including coordinated up- and down-regulation of genes related to the metabolic pathways of reproduction, nutritional dynamics, defensive system, and cell death. These data provide key information on several pathways that could be behind the lethal and sublethal effects observed in aphids feeding on *B. bassiana* colonized or primed plant tissues, establishing a robust foundation for future investigations aimed at unravelling the genetic basis of the role of entomopathogenic fungi as multipurpose microorganism for sustainable agriculture.

Keywords: entomopathogenic fungi, endophyte, *Aphis gossypii*, transcriptomes RNA-seq, ISR, SAR, DEG

A mycoviral infection drives ecological fitness of the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuill.

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Fungi can have mycoviral infections that either have not detectable effect on the infected fungal strain or cause drastic changes in their fungal hosts that are well known in fungi of medical, veterinary and phytopathogenic importance. Regarding entomopathogenic ascomycetes, there are several references on the modification of their virulence by infection with single-stranded RNA (ssRNA) or double-stranded RNA (dsRNA) viruses. It has been found that the virulence of the EA *Beauveria bassiana* can be enhanced by the presence of a virus (mycovirus-hypervirulence), a remarkable fact since most of them are asymptomatic or reduce virulence (hypovirulence). However, there is scarce information, if any, on the possible adaptative effect of these viral infections on the entomopathogenic fungal strain response to key environmental abiotic and biotic stresses that are key factors either for propagule depletion and inactivation or for reducing the infectivity and virulence of these fungi. Considering that biocontrol solutions based on entomopathogenic fungi should be based on the use of environmentally competent fungal strains for the subsequent mycoinsecticide development, information on the possible impact of mycovirus infection on this key fungal behavior is crucial. In the present work, important first ever evidence is provided on the key role of a *Beauveria bassiana* mycovirus infection on improving the environmental competence of this entomopathogenic ascomycete and the results are discussed in terms of developing a widely used biotechnological tool for deploying environmentally competent entomopathogenic fungal strains.

Keywords: temperature, water activity, osmotic stress, UV-B radiation, antagonism

Potential of entomopathogenic nematodes and entomopathogenic fungi against coconut rhinoceros beetle in Hawaii

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Coconut rhinoceros beetle (CRB, *Oryctes rhinoceros*) is a large scarab beetle native to Southeast Asia and a serious pest of palm species, most notably coconut and oil palms. CRB adults damage palms, particularly younger ones, by boring into the center of the crown, injuring the young, growing tissues and feeding on the sap. In Hawaii, CRB was first confirmed on Oahu in December 2013. Since then, USDA, Hawaii Department of Agriculture (HDOA), University of Hawaii at Manoa, Hawaii Invasive Species Council, and other entities launched the CRB eradication program, the largest invasive species eradication program in Hawaii's history. This presentation reports our latest research updates on biological control of CRB. Due to the strict regulations on importing biological control agents into Hawaii set by HDOA, our approach is to collect locally occurring entomopathogenic nematodes (EPNs) and entomopathogenic fungi (EPF), and then screen them for effective strains in both lab assays and field trials. We collected over 20 EPN strains and over 60 EPF strains from various landscape sites on Oahu. Based on our multiple lab assays, we identified one EPN strain that caused > 60% mortality of CRB larvae, and several EPN strains that caused > 30% mortality of CRB larvae. We also identified multiple EPF strains that caused > 70% mortality of CRB larvae in our lab assays. Based on our subsequent field experiments, several *Metarhizium* strains resulted in > 40% mortality of CRB larvae, and the commercial *Beauveria* strain caused almost 70% mortality of CRB larvae in field conditions. Larger-scale EPN and EPF field trials will be conducted as soon as permission from HDOA is obtained. An IPM program including both biological and chemical controls is being actively developed to control CRB in Hawaii. Most up-to-date research results will be presented at the 2024 SIP Annual Conference.

Innovative solutions to control *Asproparthenis punctiventris* with the entomopathogenic fungus *Metarhizium brunneum*

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The sugar beet (*Beta vulgaris*) provides the basis for sugar production in Central Europe and must therefore be protected. It is well documented that *Metarhizium* spp. can successfully infect the sugar beet weevil *Asproparthenis punctiventris.* Our main focus was to evaluate different application approaches of the entomopathogenic pathogenic fungus (EPF) and determine the most effective means of targeting the pest. The approaches included applications on soil, leaves, or spray of adult weevils using granular and liquid formulations based on the EPF strain *M. brunneum* Ma43 and were tested in field trials. For further support weevil control, trap ditches treated with the fungus were installed to limit the migration of the pest. At chosen locations in various climatic regions of Lower Austria, the abundance of *Metarhizium* in soil and from leave surface after the application of Ma43 formulations was evaluated and the sugar beet weevil adults were assessed for mycoses. When isolated from the pest, *Metarhizium* strain identity was assessed using microsatellite marker analysis. The weevils were highly susceptible to the production strain Ma43 in laboratory bioassays. Even in the field trials, infection rates up to 20 % were recorded with repeated or multiple applications of the fungal formulations. While the production strain was not isolated from the soil prior to treatment, more than 50% of the isolates obtained from soil after application were identified as *M.*

brunneum Ma43. For sustainable pest control, the combination of repeated treatments as well as the use of different control methods (e.g. trap ditches, alternative biological control agents, …) is the most effective way to maintain sugar beet cultivation. The latest trial results will be presented and discussed.

CONTRIBUTED PAPERS **MC-11-STU**

Potential of endophytic *Beauveria bassiana* for the management of *Coraebus undatus* (Coleoptera: Buprestidae) in cork oak forests.

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Coraebus undatus, also known as the black-banded wood borer beetle, is a prominent cork oak tree pest in the Mediterranean forests. Damage is due to the larval stage that excavates subcortical feeding galleries on the phellogen layer threatening the regenerative capacity of cork and producing a cork depreciation. The two-year endophytic larval development and the lack of initial infestation indications make detection challenging. Currently, general forest management is the primary containment measure, as specific control methods have not been developed due to restrictions on insecticide use in forest ecosystems. In the frame of a recent outbreak of *C. undatus* in Sardinia (Italy), different bio-based management approaches have been explored. Among these, the assessment of the entomopathogenic potential of fungi isolated from larvae collected on infested plants led to the isolation of a novel *Beauveria bassiana* strain. The biological properties of this fungus were studied in the laboratory on the coleopteron model *Tenebrio molitor*, and on laboratory-reared larvae of *C. undatus*. These studies highlighted a significant and dose-dependent insecticidal action by contact, reaching 100% mortality at the highest concentrations assayed. To assess the ability of the fungus to enter plant tissues, potentially reaching xylophagous larvae, experiments were conducted to assess the endophytic properties of the fungal isolate employing *Quercus* sp. and *Phaseolus* sp. seedlings. Finally, the entire genome of the fungus was sequenced and analysed to gain insights into its overall entomopathogenic properties. Everything considered, this approach shows promises for the management of *C. undatus* in forests.

CONTRIBUTED PAPERS **MC-12**

Comparative genomics study on commercial strains of *Beauveria bassiana*: how genetic variability affects insecticidal activity.

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Introduction. *Beauveria bassiana* represents one of the most common examples of biocontrol. Its biocidal activity has been demonstrated on various species of insects, representing an actual alternative to the use of chemicals in agriculture. However, many studies reported the remarkable genetic variability occurring within this species, providing evidence of how the insecticidal activity of *B. bassiana* is strictly strain-dependent. Addressing the need to further clarify the mechanisms involved in this specific microbe-host interaction, we compared the strains ATCC74040, GHA and PPRI5339, used as active ingredients of commercial products, through a combined approach including bioassays, comparative genomic and transcriptomic analyses.
Results. The insecticidal activity, tested on the cotton aphid *Aphis gossypii*, was significantly different in the three strains. The mortality rate, 10 days after the treatment, was 73.8%, 55.7% and 16% for insects treated ($2.3x10^4$ conidia/ml) with ATCC74040, GHA and PPRI5339 respectively. RNA-seq on treated aphids resulted in 382959 (ATCC74040), 461 (GHA) and 1031 (PPRI5339) total reads mapped to the genome of *B. bassiana,* corresponding to 3563, 2 and 4 differentially expressed genes (DEGs). Analysis on DEGs, indicates that infectious mechanisms and mycelial growth as some of the most represented metabolic processes. Also, we identified the genes that mostly contribute to the genetic variability within the considered strains. Among the many, for its relevance, it must be noticed mycotoxin biosynthesis as one of the most represented biological processes.
Conclusions. The results allow to conclude that only the strain ATCC74040 has an early insecticidal activity against *A. gossypii*. The genetic diversity and the different gene expression profiles that characterizes the three strains might reasonably explain such differences. Ongoing experiments and analyses are expected to provide further details.

CONTRIBUTED PAPERS **MC-13**

Strategising the all-season usage of *Hirsutella thompsonii* [ICAR–NBAIR–MF(Ag)66] to manage the broad mite in mulberry

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The mulberry grown for sericulture in southern India has been under relentless attack by the broad mite, *Polyphagotarsonemus latus*. In the absence of efficacious and silkworm-safe synthetic pesticides, farmers often resort to inappropriate or dubious substitutes expecting to control this tarsonemid species, which is also a pest of several other economically important crops. Meanwhile, our research is focussed on biological control, which is a worthwhile alternative to chemicals in this unprecedented situation. Over thirty back-to-back field trials since August 2019 have demonstrated the capability of the acaropathogenic, ophiocordycipitaceous fungus *Hirsutella thompsonii* [ICAR–NBAIR–MF(Ag)66] either as a stand-alone treatment or as a pivot in an integrated package for broad mite management in mulberry. Favourable results were obtained when the predatory phytoseiid mite *Typhlodromus* (*Anthoseius*) *transvaalensis* and the botanical biopesticide azadirachtin were included in various sequences with a mycelial–conidial liquid formulation of the fungus. Auxiliary biocontrol agents will be evaluated in our upcoming field trials to develop new sequences of weekly treatments for the broad mite, especially to strategise the all-season usage of the fungus. One of the challenges, however, is to consider the threat from other regular pests of mulberry, such as a couple of mealybug species, a leaf roller and multiple thrips species, mostly *Pseudodendrothrips darci*. The aim is to give the farmers more leeway to use biocontrol.

CONTRIBUTED PAPERS **MC-14**

Implementing indirect effects of entomopathogenic fungi in biocontrol of spider mites

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Applications of entomopathogenic fungi (EPF) as biocontrol agents traditionally rely on their direct effects on the target host through infection, resulting in host mortality. However, several EPF species can also interact with insects and mites indirectly where a third organism, e.g., a plant, is mediating the interaction. Such indirect interactions may have profound effects on the pest populations and hence also impact outcomes of biological control efforts. Thus, EPF can potentially modify host plant physiology to cause indirect effects on pests feeding on the plant. We evaluated the biocontrol effects of spider mites, *Tetranychus*

urticae, in strawberry production in Denmark, where the EPF *Metarhizium brunneum* was applied to the growing substrate as biocontrol strategy against soil-borne insect stages. The below-ground *M. brunneum* application led to reduced spider mite population growth on above-ground parts of strawberry plants. Spider mites were also targeted with the predatory mites *Neoseiulus cucumeris* as macro-biological control agent. The combined effect of *M. brunneum* and *N. cucumeris* was additive in climate room experiments indicating a potential of integrating indirect effects of EPF in biocontrol programs against *T. urticae*. Further, the strategy was tested in two commercial strawberry tunnels during one growing season from April till September with a single application of *M. brunneum* at the start of the season and regular releases of *N. cucumeris*. The above-ground arthropod pest communities were monitored regularly, showing that the plots with combined biocontrol applications showed delayed pest population build-up or no difference in comparison to single application or control plots. In September, *M. brunneum* successfully infected insect bait larvae in substrate samples. Indirect effects of EPF mediated by plants can be implemented in biocontrol programs when the EPF is applied for specific biocontrol of e.g., below-ground pest stages.

CONTRIBUTED PAPERS **MC-15**

Constitutive and inducible tomato defenses contribute to *Bacillus thuringiensis* lethality against *Spodoptera exigua*

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Plants have developed a wide array of constitutive and inducible defenses to protect themselves from the attack of insect herbivores, such as caterpillars. These defenses can either directly affect the insect pest due to their antifeedant, deterrent or toxic properties, or participate in the recruitment of natural enemies of the herbivore, like parasitoids or predators. However, the impact of plant defenses on other trophic levels, such as entomopathogens, needs further investigation. In this work, we assessed the influence of constitutive and inducible foliar defenses of tomato on the lethality of the bacterial entomopathogen *Bacillus thuringiensis* (Bt) against the herbivorous caterpillar *Spodoptera exigua*. First, we explored the effect of constitutive defenses from 55 tomato genotypes, including fifteen wild tomato species and forty cultivated tomato varieties (*Solanum lycopersicum*), on *S. exigua* larval growth and susceptibility to Bt. Then, we selected six cultivated varieties with contrasting levels of resistance to *S. exigua*, which were treated with the phytohormone methyl jasmonate (MeJA) to mimic herbivory and elicit tomato defenses. Our results showed that constitutive metabolites from wild tomato species had stronger detrimental effects those from cultivated varieties, since a lower larval growth was detected. We also found that constitutive and inducible defenses led to a lower larval growth and a higher basal mortality, which in turn was associated with an increased larval susceptibility to Bt. Untargeted metabolomic analysis of tomato leaves after MeJA elicitation confirmed a metabolic shift in the tomato metabolome and the accumulation of some bioactive compounds, with reported anti-herbivore properties, in most of the selected varieties. Overall, our findings show that both constitutive and herbivore-inducible defenses directly affect the insect pest, but also contribute to the efficacy of bacterial entomopathogens against a generalist caterpillar.

CONTRIBUTED PAPERS **MC-16**

Benefits of Baculovirus Use in IPM Strategies

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Integrated pest management (IPM) is an integral part of our current agricultural practices. Much has improved with the integrated view to control pests with a few negative side effects as possible. However, also IPM is in a constant state of change due to new goals like significant ecologisation or due to the increasing ban on chemical active ingredients. New elements and solutions are in demand to further develop the IPM. Microbials can contribute to future aims, but they have to fulfil many properties to fit into IPM-strategies. One of the main topics is the compatibility of microbials with the existing products. Baculoviruses (BV) are one example of the microbial family, which has already shown several times their potential for us in IPM. Baculoviruses bring many benefits and allow reduced use of synthetic insecticides. In addition, their unique mode of action makes them suitable for resistance management.
In several practical experiments in different crops, commercial BV products were applied in rotations and tankmixes. In an open field trial in 2020 in Germany, Madex (CpGV) was applied in an IPM strategy in rotation with Chlorantraniliprole and reduced total damaged fruits at harvest with an efficacy of 88 %. Further trials in Canada and USA confirmed better efficacy of a tankmix with Plutex (PlxyGV) compared to stand-alone application of products or the standard program. The addition of Tutavir (PhopGV) to the standard grower program led to significantly reduced fruit damage with a long-term effect after the last Tutavir treatment.
In conclusion, baculoviruses fit into many IPM applications strategies, whether in rotation or in tank mix. Individual strategies must be developed for each different microbial and crop-pest system, whereas growers needs and expectations need to be known to find the best strategy. New and successful IPM strategy trials can help growers to find trust in novel solutions in future.

CONTRIBUTED PAPERS **MC-17**

CpGV Resistance – Where we are and where to go

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Viral biocontrol agents based on Cydia pomonella granulovirus (CpGV) are a corner stone of codling moth control in organic and integrated production of pome fruits. With the emergence of different types (I-III) of resistance to CpGV products the sustainable use of CpGV products is at risk. By developing a fast, bioassay-based method to identify CpGV-resistant larvae we have established a monitoring program to support organic growers in Germany. Our screening during the last decade revealed a shift from the occurrence of type I resistance towards the spread of type II resistance, which may reflect the use of CpGV isolates from genome group E which is prone to type II resistance. Currently 66 plantations have been identified to exhibit either type I or type II resistance. With the uses of genome group B CpGV in commercial products it could be demonstrated that codling moth damage in orchards with CpGV resistance can be returned to levels below 1% infestations within 2 years. For resistance management it will be important to further exploit the natural genetic diversity of wild-type CpGVs and those to be used in commercial products, and to combine CpGV sprays with other control tools, such as mating disruption.

Chromosome-level comparison between susceptible and resistant codling moth strains to decoding the mechanism of CpGV resistance of codling moth

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Codling moth (*Cydia pomonella* L.) is a significant pest in pome fruit production and causes significant economic losses in apple, pear and walnut orchards worldwide. It is successfully controlled by the application of commercial formulations of the Cydia pomonella granulovirus (CpGV). The emergence of different types (I-III) of CpGV resistance poses a threat for biological control of codling moth. Inheritance of type I CpGV resistance is dominant and linked to the Z chromosome. Understanding the underlying resistance mechanisms is critical for future pest management strategies.. In the present study, we have applied Oxford Nanopore sequencing of susceptible (CpS) and type I resistant (CpRR1) larvae using the latest ultra-long DNA extraction methods and sequencing techniques. Our *de novo* assembly using the Flye software pipeline generated a high quality genome sequence of codling moth, achieving the BUSCO score of 98.39% and 98.37% for CpS and CpRR1, respectively, which is comparable to a genome sequence *of C. pomonella* published in 2019. The Z chromosomal sequence and annotation of CpS and CpRR1 were compared. These genetic differences are the bases for further screening and genetic analysis of laboratory backcrosses and field individuals with the aim to locate the position of type I resistance candidate markers on the Z chromosome. This advancement in sequencing technology facilitates the development of insect chromosomal genetics and evolution as well as the study of codling moth resistance.

Fungal Entomopathogens for pest biocontrol in multitrophic approaches

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Beneficial micro-organisms associated to plants either as root colonizers or endophyte, known as hypocrealean entomopathogenic fungi (HEF), constitute new opportunities for managing crop pests and diseases. Plants, insects, and HEF are part of agroecosystems, where underground and aboveground trophic interactions play a key role in shaping the relationships between these players. Whereas the role of HEF in underpinning these interactions has recently been reported, there is little information about (1) HEF-mediate pest resistance in the plant, (2) their direct and indirect actions against insect pests of different orders, (3) their effect on aphid-borne diseases, and (4) on higher trophic level occurring between pests and their entomophagous beneficials. Here, we provide several recent results from different research projects performed on different insect models. Some HEF of the genus *Metarhizium and Beauveria* isolated from agricultural field in Belgium and Democratic Republic of Congo have been tested against sap-sucking insects such as *Myzus persicae*, *Macrosiphum euphorbiae, Sitobion avenae aphids and Halyomorpha halys* bug but also chewing insect pest, namely *Spodoptera frugiperda*. Beside the evaluation of the direct effect of the tested fungal isolates on these insect pests, the endophytic actions of these treatments have been also assessed in different host plants namely sugar beet, tomatoes, wheat, faba bean, and maize. Moreover, the effect of these fungal treatments on beneficial insect, *Episyrphus balteatus* have been also evaluated. New sustainable and complementary strategies are proposed to cope with important insect pests based on the potentialities of entomopathogenic fungal strains as bio-insecticides displaying direct and indirect effects through host plants.

Prospects of NoVil (*Metarhizium robertsii* strain CPD006) in controlling *Frankliniella occidentalis, Myzus persicae* and *Tetranychus urticae* on greenhouse crops

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The green peach aphid (GPA), *Myzus persicae,* the Western flower thrips (WFT), *Frankliniella occidentalis,* and the two-spotted mite (TSSM)*, Tetranychus urticae,* are among the major arthropod pests of vegetable and ornamental plants grown in greenhouses. Three trials each were conducted between 2020 and 2023 to evaluate the potential of NoVil EC, a *Metarhizium robertsii* strain CPD006-based biopesticide, under development, for the control of GPA on pepper, WFT on cucumber and TSSM on tomato plants. NoVil was applied at low concentration of $6.7x10^8$ CFU/L (NoVil-LC) and high concentration of $1.3x10^9$ CFU/L (NoVil-HC) against GPA and TSSM while at the low concentrations of $1x10^9$ CFU/L (NoVil-LC) and high concentration of $1.7x10^9$ CFU/L (NoVil-HC) against WFT. BotaniGard®, Lalguard M52 OD and Trounce (potassium salts of fatty acids and pyrethrins) were used as industry standards and applied at the recommended concentrations. Control treatments consisted of NoVil formulation without fungal spores. Treatments were applied three or four times for each trial and were arranged in a randomized complete block design with four replicates. Plants were artificially infested, and target pests allowed to multiply for 10-15 days before the first applications of treatments. Overall, both NoVil EC rates performed as well as or better than industry standards. In addition to reducing pest population reduction, NoVil treatments had significant negative effects on population growth of GPA as compared to the control. NoVil can be therefore considered as a potential biopesticide candidate.

Api-vectoring of *Beauveria bassiana* for thrips control in strawberry tunnels

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Thrips infestations are becoming an increasing problem in tunnel production of strawberries, particularly early in the season, and the growers have limited control options against these pests. Application of *Beauveria bassiana* in the tunnels represents a promising control strategy, but it remains a challenge to target the thrips directly. We investigate the potential of vectoring *B. bassiana* (BotaniGard WP) to strawberry flowers by bumblebees (*Bombus terrestris*), which are already used in strawberry tunnels for pollination. Api-vectoring of microbial control agents is used against grey mold, while few attempts for insect pest control with this strategy have been done. We evaluate the ability of bumblebees to vector *B. bassiana* to strawberry flowers and study the establishment and persistence of the fungus. We also assess the susceptibility of thrips to *B. bassiana* in the flowers and evaluate the consequences for the bumblebees after *B. bassiana* exposure, both

individually and in groups, as well as potential contamination of the hives by *B. bassiana*. Finally, we characterize the bumblebee microbiota after exposure to BotaniGard to identify potential indicators of bee health. The project aims to establish foundations for more widespread use of api-vectoring of microbial control agents beyond disease control and to qualify risk assessment when approving use of biocontrol agents for insect pest control.

CONTRIBUTED PAPERS **MC-22**

Antagonistic potential of entomopathogenic and endophytic fungi against fusarium wilt pathogen of tomato *Fusarium oxysporum f. sp. lycopersici*

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Entomopathogenic and endophytic fungal-based biopesticides are sustainable and ecologically-friendly biocontrol agents of several pests and diseases. However, their potential in managing tomato fusarium wilt disease (FWD) remains unexploited. This study therefore evaluated effectiveness of nine fungal isolates against tomato fusarium wilt pathogen, *Fusarium oxysporum f. sp. Lycopersici* (FOL) *in vitro* using dual culture and co-culture assays. The efficacy of three potent endophytes that inhibited the pathogen *in vitro* was assessed against FWD incidence, severity, and ability to enhance growth and yield of tomatoes *in planta.* The ability of endophytically-colonized tomato plants to systemically defend themselves upon exposure to FOL were also assessed through defence genes expression using qPCR. *In vitro* assays showed that endophytes inhibited and suppressed FOL mycelial growth better than entomopathogenic fungi (EPF). Endophytes *Trichoderma asperellum* M2RT4, *Hypocrea lixii* F3ST1, *Trichoderma harzianum* KF2R41, and *Trichoderma atroviride* ICIPE 710 had the highest (68.84-99.61%) suppression and FOL growth inhibition compared to EPF which exhibited lowest (27.05-40.63%) inhibition rates. Endophytes M2RT4, F3ST1 and KF2R41 colonized all tomato plant parts. During the *in-planta* experiment, endophytically-colonized and FOL-infected tomato plants showed significant reduction of FWD incidence and severity compared to non-inoculated plants. In addition, these endophytes contributed to improved growth promotion parameters and yield. Moreover, there was significantly higher expression of tomato defence genes in M2RT4 colonized than in un-inoculated tomato plants. These findings demonstrated that F3ST1 and M2RT4 which were initially proved to be effective against tomato leafminer and whitefly, are also effective biocontrol agents against FWD and could sustainably mitigate tomato yield losses associated with fusarium wilt, *Tuta absoluta* and *Trialeurodes vaporariorum.*
Keywords: Fusarium wilt, endophyte, phytopathogen suppression, growth inhibition, defence gens expression

CONTRIBUTED PAPERS **MS-1-STU**

Data mining reveals diversity and host spectrum of cryptic microsporidian parasites across the Panarthropods

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Microsporidia, a vast phylum of obligate intracellular parasites classified as early branching Fungi, exhibit a remarkable ability to infect a wide array of hosts, including over 220 genera of both vertebrates and invertebrates. Jaroslav Weiser once said “my experience in distribution of microsporidia of invertebrate hosts indicates that there may be a microsporidian in every living invertebrate”. To get an idea of the extent of the vast microsporidian diversity, we employed a data mining strategy to extract valuable insights from publicly available Panarthropod genomic and transcriptomic projects. In total, 494 species across 478 genera and 35 orders of panarthropods were retrieved as ‘potentially infected’ with expected infection patterns. This not only underscores the ubiquity of these parasites across the Panarthropoda phylum, but also reveals potential tissue tropism, as well as environmental diversity with globally distributed host samples ranging from the wild to laboratory settings, including an insect cell line. Furthermore, we constructed a multi-protein phylogeny using proteins extracted from the ‘parasitised’ assemblies and reference genomes. The resulting phylogenetic analysis not only confirmed the existence of established microsporidia clades and their typical hosts but also unveiled potential novel clades. This study underscores the significance of data mining approaches in conjunction with traditional field studies, offering a promising avenue to elucidate the ecology and host spectrum of these often-overlooked parasites.

CONTRIBUTED PAPERS **MS-2**

Lithium Chloride reduces *Vairimorpha (Nosema) ceranae* infection in honey bees

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Vairimorpha (Nosema) ceranae is a microsporidian parasite that infects honey bees, causing individual pathology and contributing to colony disease. *V. ceranae* infection has traditionally been treated with the MetAP2 inhibitor fumagillin in North America, but the efficacy, safety, and availability of this drug is uncertain, making alternative therapeutic options highly desirable. One strategy for discovering novel anti-*Vairimorpha* therapeutics has focused on testing existing beekeeping drugs already commonly used in the field for activity against microsporidia infection in bees. Currently, several chemical compounds that are used for the management of *Varroa destructor* in bees have been found to have anti-microsporidia activity, including formic acid, oxalic acid, and thymol. Lithium salts have recently been shown to be highly effective in reducing *Varroa destructor* infestation in honey bees. Here, we test whether lithium salts can inhibit *V. ceranae* infection in honey bees in cage assays and show a marked reduction in parasite load after treatment with lithium chloride. While lithium salts are widely used therapeutically in humans, their mechanism of action is still somewhat obscure. We further explore potential pathogen-directed and host-directed mechanisms through which lithium treatment inhibits *V. ceranae* infection in honey bees.

CONTRIBUTED PAPERS **MS-3**

A protease associated with microsporidian spore germination

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Nosema bombycis, the first identified microsporidia, can cause pébrine and significantly damage sericulture. When microsporidian spores undergo appropriate stimuli for germination, which involves the rupture of the polar cap and discharge of the polar tube from inside, it constitutes a crucial step in the invasion of microsporidia. Previous research conducted in our laboratory has demonstrated that subtilisin-like protease 1 (NbSLP1) is detected in the apical region of the spore wall after germination. In this study, we have clarified through immunofluorescence and transmission electron microscopy that NbSLP1 is located at the position where polar tube is extruded from germinated spores. Searching the genome database of *N. bombycis* using a subtilisin-like protease cleavage motif (RXK/RR) found in eukaryotes, spore wall and anchoring disk complex protein NbSWP16 was predicted as a potential target protein. Immunofluorescence co-localization experiments and yeast two-hybrid analysis further confirmed an interaction between NbSLP1 and NbSWP16. Detailed investigation revealed that NbSLP1 interacts with a hinge region containing a predicted cleavage site within NbSWP16 protein structure. Additionally, NbSWP16 was found to interact with other spore wall proteins such as NbSWP30, NbSWP26, NbSWP12, and NbSWP17 possibly forming an interaction network at the polar cap to maintain proper morphology of the spore. These findings suggest that interaction between NbSLP1 and NbSWP16 may facilitate the polar cap rupture necessary for the efficient ejection of polar tubes.

CONTRIBUTED PAPERS **MS-4-STU**

The diversity and co-infections of microsporidia in coexisting cryptic lineages of an amphipod crustacean: what can we learn from Nanopore amplicon sequencing?

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Microsporidia are frequent and ecologically relevant parasites of amphipod crustaceans, a keystone group in freshwater macrozoobenthos. *Gammarus fossarum*, a widespread amphipod morphospecies in Europe, is in fact a diverse species complex comprising tens of divergent lineages. While studying their ecological and evolutionary interactions in the Carpathians, we also focus on microsporidian lineage diversity and infection patterns in relation to host lineage. Over 2000 gammarids were screened for infection by PCR with microsporidian-specific primers (targeting the small subunit rRNA gene) followed by Sanger sequencing. Besides the widespread presence of common microsporidian genera known from *Gammarus* (*Nosema*, *Cucumispora*, *Dictyocoela*), numerous uncharacterized microsporidia were detected. While some may represent novel divergent clades, others are related to parasites already known from amphipods. In a detailed study of the syntopy of three host lineages, we observed strong variation in microsporidian specificity: three out of six common parasite lineages showed unbalanced infection patterns, each being absent from a different *Gammarus* lineage. These results contribute to our understanding of microsporidian diversity, infection patterns, and their potential role in amphipod ecological interactions and coexistence. However, Sanger sequencing frequently resulted in unreadable or noisy chromatograms, possibly due to co-amplification of multiple microsporidians from a single host. To test this, we used amplicon sequencing on the Oxford Nanopore platform. The resulting consensus sequences closely matched those from Sanger sequencing. Microsporidia were identified from almost all samples where sequencing originally failed, and frequent co-infections (commonly with several different microsporidia) were confirmed. The implementation of this approach is cost-effective and reveals additional parasite diversity and co-infection patterns missed by conventional Sanger sequencing.

CONTRIBUTED PAPERS **MS-5**

Molecular, Morphological identification and whole genomic sequencing of a microsporidium from cucumber moth, *Diaphania indica* (Saunders)

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Microsporidia are obligate intracellular fungi with a wide host range. In this study, the diseased larvae of cucumber moth, *Diaphania indica* (Saunders) showed microsporidiosis was confirmed. The mature spore of microsporidia was observed, and the fresh spores were oval in shape and measured 3.737 ± 0.290 × 2.079 ± 0.140 μm (length × width, n= 200). The whole ribosomal RNA (rRNA) was then sequenced. The complete rRNA genome is 4,292 bp and the orientation is LSUrRNA-ITS-SSUrRNA-IGS-5S, which shows a typical *Nosema* genus feature. Phylogenetic analysis based on the ribosomal RNA showed that the microsporidia belong to the genus *Nosema* and closely related to *Nosema mylitta*. Besides, observation of the ultrastructure by transmission electron microscope (TEM) showed the presence of characteristic structures such as polar tubules, merogony, and sporogony typical features of microsporidia. To further investigate the cucumber moth microsporidium, whole genome was sequenced by Oxford Nanopore Technology (ONT). The whole genome of this microsporidium is 21,410,320 bp (21.4 Mbp) with an average coverage of 33.8x. The largest contig and the N_{50} of assembled genome are 336 kb and 91 kb, respectively. The completeness of BUSCO score is 92.2 %, though the genome size is larger than other microsporidia in the genus *Nosema*. In the future, the Next-Generation Sequencing (NGS) data will be engaged to assemble a more complete genome, thereby bringing valuable insights into the genetic composition and potential pathogenicity of the cucumber moth microsporidium.

First record of Nosema maddoxi (Microsporidia, Nosematidae) in populations of Nezera viridula (L) and Palomena prasina (L) in Georgia

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Nezara viridula (L.) and *Palomena prasina* (L) are commonly found shield bugs in Europe and the USA. Individuals of these bugs were detected and collected in fruit orchards together with *Halyomorpha halys* (Stål) in Anaseuli, Guria region, Georgia. The bugs were dissected and examined for microsporidian infection. The fat body and gut were extracted, smears were prepared, and microscopic analysis was conducted using phase contrast microscopy at 400×. Infections were detected in both bug species, the smears were stained with Giemsa. Spores were measured, and their morphology was analysed. The mean spore size of fixed spores was 2.96 × 1.57 µm from *P. prasina* and 2.91 × 1.63 µm from *N. viridula*.
The microsporidian pathogen in both bug species has been identified as *Nosema maddoxi* by sequencing the partial small subunit (SSU) region of rDNA. The obtained sequences, which varied in length from 908 to 988 base pairs, exhibited a similarity of 99.90-100% to a *N. maddoxi* SSU consensus sequence (GenBank accession number KY783624) from a microsporidium found in *Chinavia hilaris*, the native green stink bug in North America. The *N. maddoxi* sequences obtained in this study were submitted to GenBank, and accession numbers PP646742 for the sample from *P. prasina* and PP646743 for the sample from *N. viridula* were assigned. *Nosema maddoxi* is a prevalent pathogen of *H. halys*, previously observed in populations of this bug in the same region. This is the first record of this pathogen in populations of *P. prasina* and *N. viridula* in Georgia.

A safe and effective control method against the fall armyworm with entomopathogenic nematodes

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The fall armyworm (FAW), *Spodoptera frugiperda* Smith, is a voracious pest of maize originating from the Americas. Recently, it has spread across Africa and Asia where it is causing tremendous plant damage and yield losses, thereby threatening the livelihood and food security of millions. In their attempts to control FAW, farmers have drastically increased the use of insecticides. The negative impacts of these toxins on public health and the environment have prompted calls for safer and more sustainable alternatives. Herein, we developed an innocuous gel formulation made of carboxymethyl cellulose to apply entomopathogenic nematodes (EPN) directly into the whorl of maize plants, where the caterpillars preferentially feed. In laboratory assays and in field trials in Rwanda the application of a low dose of a locally isolated EPN formulated in the gel considerably reduced FAW infestation and plant damage, translating into an increased grain yield.
We now explore ways to further increase the efficacy of the formulation. In one approach, we are testing UV protectants in the formulation to enhance EPN longevity. Another approach is to supplement the formulation with odorous compounds that influence the behaviour of caterpillars and moths. Certain compounds might encourage caterpillars to feed on the EPN-gel – as an attract-and-kill strategy – while others could discourage moths from laying their eggs on the treated plants. In addition, we are evaluating different cost-effective and more practical application approaches to meet the specific needs of both small- and large-scale maize cropping systems.
A newly funded SOR4D project from the Swiss National Science Foundation will allow us to to collaborate with *icipe* (International Centre of Insect Physiology and Ecology), Dudutec Biocontrol in Kenya and CABI to conduct large scale trials to test the efficacy of our formulation under realistic conditions and to transfer the technology to farmers across Africa.

Increasing the efficacy of Steinernema australe after fourteen generations by using volatile root signals of blueberry plants.

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The searching behavior is one of the most studied characteristics in entomopathogenic nematodes (EPNs), especially when the target pest is hidden and cryptically located. The responsiveness of the EPN Steinernema australe to volatile root signals was evaluated in 2022 against Aegorhinus nodipennis (Coleoptera: Curculionidae) larvae after six generations, resulting in a 20% increase in efficacy in blueberry and sarsaparilla orchards when the compound 2-carenne was used as an odor stimulus. One year later, the 6th generation infective juveniles (IJs), maintained at 4°C, were used to continue the selection process. Generations 7 to 14 were conducted under laboratory and greenhouse conditions using a modified T-shaped olfactometer. The objective was to determine the effect of time on the selected IJs to demonstrate if the efficacy of selected IJs may increase after fourteen generations of selection. IJs that followed the stimulus (2-carenne) faster at 30 cm depth into a vertical olfactometer were collected. The number of IJs collected in each arm of the olfactometer was considered the variable and analyzed using ANOVA. The results showed that after 14 generations, IJs of S. australe increased their efficacy by 12% compared with those from the 6th generation and increased by 32% compared to those from the original stock. After one year, the transition from the 6th to the 7th generation showed an increase of 5% efficacy under greenhouse conditions; however, the number of IJs that reach the stimulus faster decreased three times compared with those from the 6th generation. These results not only showed a potential tool to increase the efficacy of native EPNs that show promissory control on native pests worldwide but also demonstrated that selected IJs can be maintained at 4°C for several months without losing their acquired feature.

Integrating host plant resistance with biocontrol: silicon supplementation and entomopathogenic nematodes to control major turfgrass insects

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This presentation elucidates the combined effects of silicon supplementation and entomopathogenic nematodes (EPNs) on controlling three major turfgrass insects: black cutworm (*Agrotis ipsilon*), white grub (*Anomala orientalis*) and annual bluegrass weevil (*Listronotus maculicollis*), with a focus on the efficacy of EPNs (*Steinernema carpocapsae* and *Heterorhabditis bacteriophora*) when applied in tandem with Si fertilization. Silicon (Si), a beneficial nutrient

for plants, can provide multiple benefits to turfgrasses, including resistance to biotic and abiotic stresses. Grasses are high accumulators of silicon, which can account for up to 10% Si of their shoot dry weight. Si is taken up by plants as orthosilicic acid and deposited in cell walls and cell lumens as amorphous silica gel or phytoliths. Si deposition makes tissues abrasive and tougher, which is difficult for insects to consume and digest. Recently, feeding on Si-supplemented plants has been shown to compromise anti-predator defenses in insects including cellular and humoral immunity. It remains unknown, however, how such impaired immunity affects insect susceptibility to biocontrol agents such as EPNs. We conducted a series of laboratory and glasshouse experiments to investigate the effects of Si supply to different turfgrasses (i.e., annual bluegrass, creeping bentgrass and perennial ryegrass) in combination with the application of EPNs (*S. carpocapsae* or *H. bacteriophora*) on controlling these three insect pests. Our observations to date suggest that Si fertilization could support plant resistance against these turfgrass insects, particularly against cutworms, and could enhance insect susceptibility to entomopathogenic nematodes, which may facilitate insect biocontrol.

CONTRIBUTED PAPERS **V-1**

(**Abstract for Early Career Award Presentation**)

An ancient and conserved viral entry mechanism mediated by PIFs

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Most enveloped animal viruses utilize one or two proteins for binding, fusion and entry into host cells. In contrast, baculoviruses encode at least 10 *per os* infectivity factors (PIFs) that are essential for midgut infection. Interestingly, PIF gene homologs could also be found in a large number of nuclear arthropod-specific large DNA viruses (NALDVs), which encompass members of over 5 different virus taxa. The common ancestor of these NALDVs may have been arisen about 500 million years ago. We and other research groups previously reported that nine of ten PIFs (PIF0 – PIF9, except PIF5) form a ~ 500 kDa protein complex in baculovirus, and showed that integrity of the PIF complex is essential for virus infection. We recently unraveled a similar PIF complex of ~700 kDa for white spot syndrome virus (WSSV). The WSSV PIF complex consists of at least 8 viral proteins, including PIF0-4, VP124, WSV021, and WSV136, and is involved in oral infection. Thus, a functional PIF complex probably has already existed in the common ancestor of NALDVs, highlighting the ancient and conserved viral entry mechanism mediated by PIFs. However, the mode of action of the PIF complex and the role of PIF5 in the infection process is still enigmatic and the subject of my future research. Our recent work found that PIF0 undergoes a conserved cleavage through NALDVs, and we revised the cleavage model for baculovirus AcMNPV PIF0 that both occlusion body and host midgut derived protease cleave PIF0 in the middle region to expose a potential fusion peptide. Our preliminary data suggest that PIF0 might function as a class I fusion protein. Structural investigations of individual PIFs as well as the complex will be crucial to further understanding of the entry mechanism.

CONTRIBUTED PAPERS **V-2-STU**

The "nudist" viruses from prehistoric times: Data-driven discovery of novel nudiviruses from ectoparasitic insects prompts a taxonomic and evolutionary re-evaluation within the family *Nudiviridae*

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The nudiviruses (family: *Nudiviridae*) are large dsDNA viruses that infect a variety of insects and crustaceans, and have most recently been identified from ectoparasitic counterparts (fleas and lice). This virus family was created in 2014 and has since been expanded via the discovery of multiple novel viral candidates or accepted members, sparking the need for a new taxonomic and evolutionary overview.
Using current information (including data from public databases), we construct a new comprehensive phylogeny, encompassing 17 concatenated nudivirus core genes from 49 nudivirus species, and further investigate core gene synteny in complete nudiviral genomes. Best-fit substitutions models for each core gene were determined separately to infer a phylogenetic virus tree based on partitioned molecular data. Additionally, by combining the branch length information of the phylogeny and fossil records of the related endogenous bracoviruses as calibration points, we generated a molecular dating tree (MDT) to estimate main evolutionary events of nudiviruses and related virus groups, i.e. bracoviruses and baculoviruses. Finally, we provide a comprehensive overview of confirmed and putative nudiviruses and their geographical distributions, as well as details about newly emerging ecological niches and societal relevance these new members of *Nudiviridae* might associate with.
Our phylogenetic and gene synteny analysis supports a new taxonomic structure of the *Nudiviridae* by suggesting two new virus genera (Zetanudivirus and Etanudivirus), from ectoparasitic lice. The MDT estimates the first emergence of nudiviruses over 280 million years ago (Mya) with insect-infecting nudiviruses seemingly emerging before crustacean-infecting nudiviruses, including their potential evolution and diversification in lice on early birds in the Cretaceous period. Studies are required to unravel the morphology and pathology of these new nudiviruses, which may in turn reveal their ecological importance.

CONTRIBUTED PAPERS **V-3-STU**

The microbiome of continuous Asian citrus psyllid cell lines

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The Asian citrus psyllid (ACP), *Diaphorina citri*, vectors the bacterial causative agent of a devastating disease of citrus plants called huanglongbing or citrus greening disease. We have developed two continuous cell lines,Dici1 and Dici3, derived from ACP ovarian tissue, which provide groundbreaking tools for investigation of the cell biology, bacteria, and viruses associated with this pest. While the Dici3 cell line has consistently been stable, the Dici1 cell line has frequently crashed.
To determine whether viruses or bacteria are present that might affect the utility of these cell lines, we sequenced the transcriptomes. Host-derived transcripts were filtered out, and the remaining sequences were annotated using MMSeqs2. Transcripts from Wolbachia and a *Diaphorina citri* reovirus were identified in Dici1. The presence of Wolbachia and the reovirus were confirmed by confocal microscopy with

a fluorescent anti-Wolbachia antibody and by RT-PCR, respectively. Additionally, the genome completeness of Wolbachia was assessed using the Benchmarking Universal Single-Copy Orthologue (BUSCO) tool. Transcripts from the reovirus were identified at low levels in the transcriptome of the Dici3 cell line but were not detected by RT-PCR. No transcripts from other bacteria, or from protozoa or archaea were detected. The Dici3 cell line has been used to study the replication of a subset of ACP viruses, demonstrating the potential utility of these continuous ACP cell lines.

CONTRIBUTED PAPERS **V-4-STU**

The isolation, identification, and characterisation of a novel *Alphabaculovirus* isolated from *Serrodes partita*

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In various studies, viruses that are harboured by several agriculturally important pests in South Africa, including *Plutella xylostella*, *Phthorimaea operculella* and *Thaumatotibia leucotreta*, have been isolated and biologically characterised, and some are now commercially available as biopesticides. Changing climatic conditions or changes in farming practices may result in changes in the status of certain pests in different agricultural crops. To safeguard crop production in South Africa, continued bioprospecting for novel biological control options, particularly viruses, against major, minor, and emerging insect pests is important. The aim of this study was therefore to isolate, identify, and characterise novel viruses from various insect pests. To date, infected larval cadavers from the fruit piercing moth, *Serrodes partita*, were obtained. Baculovirus occlusion bodies have been purified from the larval cadavers enabling the successful extraction of genomic DNA, which was used for PCR amplification of the targeted *polyhedrin*/*granulin*, *late expression factor 8* and *late expression factor 9* genes. Sanger sequencing of the target regions was conducted, followed by phylogenetic analysis. The virus was identified as an *Alphabaculovirus*, grouping closely to Lymantria dispar MNPV and Lymantria xylina NPV, with Kimura two-parameter distance matrices indicating this virus to be a novel species, hereafter referred to as SepaNPV. Whole genome Illumina sequencing was conducted followed by *de novo* assembly to reassemble the complete genome sequence. Preliminary results indicate a genome size of 129953 bp, with a GC content of 54.9 %, and approximately 172 ORFs. Infection assays were conducted to test the host range of SepaNPV against three potential host species, however, none were found to be susceptible to infection. Further host range testing and bioprospecting for other potential novel viruses is underway.

CONTRIBUTED PAPERS **V-5**

Untangling the strings of the puppet master: investigating the role of biogenic amine signaling cascades in AcMNPV-infected *Spodoptera exigua* caterpillars

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Parasites' modifications of host behaviour are detected in a range of systems. The baculovirus Autographa californica multiple nucleopolyhedrovirus (AcMNPV) alters the behaviour of caterpillars of the beet armyworm, *Spodoptera exigua,* into a hyperactive state after infection and the viral protein tyrosine phosphatase (PTP) is required for this expression of hyperactivity. We infected early third instar caterpillars with Wildtype (WT) AcMNPV or with AcMNPV mutants either expressing a catalytically inactive PTP or no PTP, to further investigate the potential role of biogenic amine signaling cascades in AcMNPV-PTP induced behavioural manipulation at different days post-infection. We aimed to investigate the gene expression of actors involved in the biogenic pathways to these amines and tyramine by analysing the gene expression of precursor enzymes, rate-limiting enzymes, and the receptors in these major biogenic pathways. We also measured the concentration in the haemolymph of the three major biogenic amines in invertebrates: serotonin, dopamine, and octopamine. Results showed that the genes for most of the precursors, rate-limiting enzymes, and receptors were highly expressed on the first day post infection. Moreover, the gene encoding for the serotonin receptor 5HT-7 was upregulated in AcMNPV WT-infected larval heads compared to other infections. In addition, a similar trend was seen for the gene encoding the tyramine receptor TAR-1. Furthermore, serotonin and dopamine concentrations were influenced by the days post infection and the viral infections. These results suggest that the baculovirus AcMNPV hijacks 5HT-7 and TAR-1 dependent signaling pathways to induce hyperactivity. In addition, our results demonstrate the impact of the host's developmental stage on AcMNPV-induced host manipulation, highlighting the complex influence of these factors.

CONTRIBUTED PAPERS **V-6**

AcMNPV P74 is cleaved at R325 and R334 by proteinases of both OB and BBMV to expose a potential fusion peptide for oral infection

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Baculoviruses enter insect midgut epithelial cells via a set of occlusion-derived virion (ODV) envelope proteins called *per os* infectivity factors (PIFs). P74 of Autographa californica multicapsid nucleopolyhedrovirus (AcMNPV), which was the first identified PIF, is cleaved by an endogenous proteinase embedded within the occlusion body during *per os* infection, but the target site(s) and function of the cleavage have not yet been ascertained. Here, based on bioinformatics analyses, we report that cleavage was predicted at an arginine and lysine (R/K)-rich region in the middle of P74. A series of recombinant viruses with site-directed mutants in this region of P74 were generated. R325 or R334 was identified as primary cleavage site. In addition, we showed that P74 is also cleaved by brush border membrane vesicles (BBMV) of the host insect at R325 or R334, instead of R195, R196, and R199 as previously reported. Simultaneous mutations in R195, R196, and R199 lead to instability of P74 during ODV release. Bioassays showed that mutations at both R325 and R334 significantly affected oral infectivity. Taken

together, our data show that both R325 and R334 of AcMNPV P74 is the primary cleavage site for both occlusion body endogenous proteinase and BBMV proteinase during ODV release and are critical for oral infection.

Characterization of proteins packaged into *Venturia canescens* Virus-Like Particles and their transcriptional control

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Venturia canescens wasps (Ichneumonidae) produce Virus-Like Particles (VLPs) in their ovaries, which are injected into lepidopteran hosts during wasp parasitism. VLPs are derived from stably-inherited nudivirus genes integrated into the wasp genome. VLPs lack nucleocapsids and instead package wasp-derived proteins (VLP1, VLP2, and VLP3). Specific removal of VLPs via RNAi targeting viral genes results in eggs that are recognized and attacked by the host immune system, demonstrating that VLPs are essential for successful parasitism by wasps. Predicted roles for viral genes include a viral RNA polymerase, and proteins involved in viral infectivity. Here, we aim to characterize VLP components and use experiments to understand the molecular mechanisms underlying their replication in wasp cells. Although the viral components of VLPs were described previously, no proteomic data for the non-viral constituents of VLPs exists outside of VLP1-3. We purified VLPs with ultracentrifugation and profiled their proteome, identifying 29 virus- and 125 wasp-derived proteins. 54 wasp-derived proteins were detected at lower levels and are encoded by genes expressed in many tissues, suggesting that these proteins have housekeeping roles and are not important VLP components. The remaining 71 proteins were present at high abundance and consisted of proteins common in wasp venom including Rho-GAP family members, proteases, and serpins, as well as the VLP proteins. We asked whether transcription of genes encoding VLP components was affected by knockdown of the viral RNA polymerase. While viral genes were strongly affected (>8-fold reduction with knockdown), genes encoding VLP components were more modestly affected (1.3-8 fold reduction, 53% of genes) or not affected (42% of genes). These data suggest that the viral transcription system either directly or indirectly impacts transcription of wasp-derived proteins packaged into VLPs. Evidence for the involvement of both wasp and viral proteins to produce VLPs provides valuable information about the evolution of biological complexity through the acquisition of viral genes by insects.

Genetic diversity of RNA viruses infecting invertebrate pests of rice

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Invertebrate species are a natural reservoir of viral genetic diversity, and invertebrate pests are widely distributed in crop fields. However, information on viruses infecting invertebrate pests of crops is limited. In this report, we describe the deep metatranscriptomic sequencing of 88 invertebrate samples covering all major invertebrate pests in rice fields. We identify 296 new RNA viruses and 13 known RNA viruses. These viruses clustered within 31 families, with many highly divergent viruses constituting potentially new families and genera. Of the identified viruses, 13 RNA viruses clustered within the *Fiersviridae* family of bacteriophages, 48 RNA viruses clustered within families and genera of mycoviruses. We detected known rice viruses in novel invertebrate hosts at high abundances. Furthermore, some novel RNA viruses have genome structures closely matching to known plant viruses and clustered within genera of several plant virus species. 45 potential insect pathogenic RNA viruses were detected in invertebrate species. Our analysis revealed that host taxonomy plays a major role and geographical location plays an important role in structuring viral diversity. Cross-species transmission of RNA viruses was detected between invertebrate hosts. Newly identified viral genomes showed extensive variation for invertebrate viral families or genera. Together, the large-scale metatranscriptomic analysis greatly expands our understanding of RNA viruses in rice invertebrate species, the results provides valuable information for developing efficient strategies to manage insect pests and virus-mediated crop diseases.

The haplotypic structure in baculovirus isolates deciphered by SNV linkage and machine learning

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Baculovirus isolates consist of various haplotypes, individual genomes that differ in their DNA sequence by single nucleotide variants (SNVs), insertions and deletions. However, the large size of dsDNA genomes makes it difficult to decipher their haplotypic composition. In most cases, a consensus sequence is generated from the sequence data, which masks the intra-isolate variation. Reconstruction of haplotypes from sequence data based on fragmented DNA is a bioinformatic challenge, as individual sequences (=reads) have to be computationally assigned to individual haplotypes. A promising approach to make this assignment is to map reads against a reference genome and to use SVNs as markers. In the present study, machine learning was used for a precise assignment of reads from Bombyx mori nucleopolyhedrovirus (BmNPV) and Spodoptera frugiperda multiple nucleopolyhedrovirus (SfMNPV) isolates using mixture models trained by expectation-maximization algorithms. This procedure helps to unravel the genetic complexity of virus populations, whereby reads are assigned a statistical probability of belonging to a particular haplotype (=component) and are further considered for assembling haplotype genome sequences. The determination of SNV positions is based on highly accurate Illumina data, with the linkage relying on the long but less accurate Nanopore reads. The combination of both sequencing techniques provided a more accurate determination of SNV positions and comprehensive coverage of the viral genomes. However, it is important that both sequencing were performed on the same DNA.Model selection was based on Bayesian Information Criteria (BIC), while training of the mixture model resulted in improved accuracy. Despite the challenge posed by the lower accuracy of the Nanopore reads, the advanced bioinformatic processing, supported by machine learning, enabled the reconstruction of individual haplotypes and a deeper insight into the structure of baculovirus isolates.

A *cis*-acting negative regulator of baculovirus spread catalyzes natural selection of deletion genotypes in cell culture

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Natural isolates of *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) are composed of several genotypic variants. Most of these carry genome deletions of variable size in the ORF15-41 region. These genotypic variants lack biological activity *in vivo*, yet can replicate very efficiently in insect cell culture in contrast to the wild type (wt) SeMNPV which replicates poorly in insect cells. The question is which genetic element (gene) of the SeMNPV genome is involved in or regulates the slow cell-to-cell spread of wt SeMNPV in insect cells.
To study this issue bacmids containing the complete SeMNPV genome (SeBac10) and a natural genotypic variant (SeBac72) were generated and functionally analyzed. SeBac72 displayed much more efficient viral spread in *S. exigua* cells as compared to SeBac10, suggesting that one or more gene(s) in the deleted region of SeBac72 negatively affect viral spread of SeMNPV in cell culture. Complete genome sequencing of SeBac72 revealed a 9.5 kb deletion encompassing ORF16-28. SeBac10-derived bacmids with combined or individual ORF16-28 knockouts were constructed to assess viral spread in *S. exigua* cells. SeMNPV ORF28 (*se28*) was identified as the responsible genetic element preventing successful spread of genome-length SeMNPV in cultured cells, which explains why *se28*-deleted viral genomes or natural variants that lack *Se28* are preferentially selected in cell culture.
Unexpectedly, RNAi-based gene silencing of *se28* did not show enhanced virus spread of SeBac10. Furthermore, *se28* reinsertion into SeBac72 (at a different locus) or in the heterologous *Autographa californica* (Ac)MNPV did not show reduced viral spread. These experiments may suggest that neither the transcript nor the translated product (Se28), but rather the DNA sequence and/or the sequence topology of *se28* play a role in the reduced viral spread of wt SeMNPV in cell culture. We propose that the *se28* region is a cis-acting negative regulator of viral spread and plays a key role in the selection of genotypic variants in natural baculovirus isolates and in cell culture.

Gene flow analysis of Cnaphalocrocis medinalis granulovirus in southern China

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Cnaphalocrocis medinalis granulovirus (CnmeGV) specifically infects rice leafroller (*Cnaphalocrocis medinalis*) and has the potential to be used as a biopesticide. Since 2013, we have conducted extensive investigations on the natural prevalence of CnmeGV in southern China and found several CnmeGV populations in Guangdong and Guangxi Provinces. Genomic analysis has revealed variations in the genetic structures of the CnmeGV geographical populations. Gene flow is a fundamental evolutionary force in adaptation. In the present study, gene flow rates between these CnmeGV populations was investigated. Phylogenetic analysis of 332 CnmeGV isolates from 12 geographical populations revealed that CnmeGV can still be divided into the Zhusanjiao Plain branch and Maozhan Plain branch. The Slatkin-Maddison panmixia test showed that there is a gene flow barrier between the two plains where CnmeGV populations are located. Population genetic differentiation analysis using the fixation indices demonstrated that gene flow in CnmeGV is weak. However, there is a difference in gene flow rates between CnmeGV populations within each plain. Admixture analysis revealed genetic mixing between CnmeGV populations in the Zhusanjiao Plain, indicating that gene flow of CnmeGV is bi-directional. These results contribute to understanding the transmission and spread of CnmeGV and provide a theoretical basis for its application as a biopesticide in fields.

Erv family proteins are the relics of pre-cellular life

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Tracing the origins of life is a fundament scientific issue. Nucleocytoplasmic large DNA viruses (NCLDVs) and nuclear arthropod-specific large DNA viruses (NALDVs) exhibit unique biological characteristics and occupy a distinct evolutionary niche in the tree of life. In this study, the Erv sulfhydryl oxidase, a viral hallmark gene shared by NCLDV/NALDV and cellular organisms, was selected to investigate their co-evolutionary dynamics. Phylogenetic analysis indicates that NCLDV Ervs and NALDV Ervs exhibit limited evolutional relationship. Functional complementary experiment demonstrated that most Erv from NALDV and NCLDV could complement, at least partially, the function of baculovirus-Erv. Using ASFV as a representative, analysis revealed that heterologous Erv better complements the function of AcMNPV Erv in the cytoplasm and to a lesser extent its function in the nuclei. A new dimeric form of Erv was revealed in the resolved structure of Hytrosavirus-Erv, and mutational analysis of its interface suggests the significance of dimerization for the Erv activity. Additionally, the function of Erv with the dual catalytic center, found in metagenomic data, were characterized, confirming the existence of the Erv type hypothesized as an intermediate state in the Erv evolutionary hypothesis. These data demonstrate that, despite prolonged evolution and diversification, the Ervs of viruses and cellular organisms still retain some exchangeability, suggesting that Ervs are relics of pre-cellular life.

Construction of an AcMNPV minigenome by synthetic biology

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Baculoviruses produce two types of virus particles, budded viruses (BVs) and occlusion-derived viruses (ODVs), which mediate oral infection and systemic infection respectively. Some of the baculoviral genes, such as *per os* infectivity factors (PIFs), are not essential for BV production *in vitro*. Deletion of these non-essential genes from the genome would theoretically obtain a minigenome that supports BV production *in vitro*. Autographa californica multicapsid nucleopolyhedrovirus (AcMNPV) contains a genome of ~134 kb and encodes more than 150 ORFs. Previously we synthesized AcMNPV genome by three-step transformation-associated recombination (TAR) in yeast using three C level fragments (C1, C2, and C3). Recently, by using "design-build-test" strategy a synthetic virus AcMNPV-mC1-1.1 in which C1 was reduced ~17.2 kb was successfully obtained. Here, we report the reduction of C2 and C3 fragments, as well as the whole genome. By using the same synthetic strategy, AcMNPV-mC2-1.0 and AcMNPV-mC3-1.0 with 7.6 kb and 17.3 kb deletion in C2 and C3, respectively, were obtained. In addition, we established a new method

for rescuing the synthetic virus by co-transfection of three overlapping linearized C fragments into insect cells. In this way, only two steps of TAR are needed and the efficiency of synthesis and rescue is greatly improved. Using this method, an AcMNPV-Syn-Mini with ~27 kb genome deletion was finally rescued. The minigenome not only lays a foundation for the future study of baculovirus functional genome, but also provides a platform for the construction of baculoviral expression vector with large cargo capacity.

CONTRIBUTED PAPERS **V-14**

Insights into the Cell Fusing Agent Virus infection dynamics and its regulation by *Aedes albopictus* non retroviral endogenous viral elements

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The dynamic interactions between a virus and its host are critical to determine the outcomes of infection. For a successful infection, the virus should adapt strategies to enter the host cell, replicate and promote the viral progeny propagation within adjacent cells and between individuals of the host population, while manipulating the intracellular environment and evading the host immunity. In counterpart, the host evolves and diversifies its antiviral defence mechanisms. In the case of persistent infections, the continuous struggle between viruses and their hosts to maintain an equilibrium between the viral transmission and the host fitness can result in genomic signatures of this long-lasting interaction. In this context, we morphologically characterized the infection dynamics of the cell fusing agent virus (CFAV: *Flaviviridae*) in *Aedes* cells as well as its effects on the mosquito *Aedes albopictus* longevity. CFAV is an insect specific virus which has been shown to persistently infect *Aedes* spp. mosquitoes in the wild. We further provide insights into the effect of non- retroviral endogenous viral elements (nrEVEs) on CFAV-infected cells and the molecular mechanisms of the interaction CFAV-nrEVE. We show that the expression of selected *Ae. albopictus* nrEVEs can be regulated in response to the CFAV infection resulting in a reduction of the viral RNA load *in vivo*. We further used *in vitro* assays in infected cells showing that selected nrEVE affects the viral replication most probably through interfering with the CFAV replication complex.

CONTRIBUTED PAPERS **V-15**

Assessment of insect specificity and pathogenicity of RNA and DNA viruses for GMO safety evaluation

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The findings of a literature review to identify[1] and describe virus taxa specific for insects (Order *Insecta*). This information aids in the risk assessment and categorization of genetic modification ventures. Criteria were defined and used in a weight-of-evidence approach to assess the specificity and pathogenicity of putative insect-specific viruses (ISV). Viruses that are transmitted by insect vectors to vertebrates (arboviruses) and viruses of other arthropods (ticks, mites) were also excluded from the study. Endogenous viral elements and errantiviruses were also not investigated.
The taxonomy of viruses as determined by the International Committee on Taxonomy of Viruses (ICTV) and the available body of relevant literature up to December 2018 were used as a final point of reference for this study. The insect-specific virus taxa identified and assessed are listed at the level of the highest taxon, that potentially contains ISVs.
Multiple criteria were defined and assessed using a weight-of-evidence approach. Important criteria were: ISVs (i) have a (restricted) host range in insects only, (ii) do not replicate in or have any effects on vertebrates or vertebrate cells and (iii) have an unequivocal phylogenetic position. Thirty-eight taxa (13 families, 3 subfamilies, 18 genera and 3 species groups) encompassing 518 putative ISVs in total were assessed. **Eleven virus families, 1 subfamily, 16 genera and two species groups were considered insect-specific (ISV) for a total of 470 virus species.**
Viruses of six taxa, the genera *Phasmaviridae* and *Phenuiviridae*, and the *dISF group* (genus *Flavivirus*) do not yet meet all of the specific criteria for ISV status due to lack of critical information. Their phylogenetic position is equivocally sandwiched between arbovirus clades. The members of the taxa *Carmotetraviridae*, *Betairidovirinae* and *Seadornovirus* do not meet the criteria for insect specificity, as they can replicate in vertebrates or vertebrate cells.
[1] CGM 2019-01 Characteristics and pathogenicity determination of insect-specific RNA and DNA viruses

CONTRIBUTED PAPERS **V-16-STU**

The perks of being a wallflower: exploration of "silent" Iflavirus infections in *S. exigua*

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Iflavirus infections in insects are often characterised by the absence of disease symptoms. Nonetheless, because of their ability of affecting the efficiency of biological control agents, their investigation is fundamental. Using two laboratory-reared colonies of the crop-pest *Spodoptera exigua* and its "silent" RNA viruses (SeIV1 and SeIV2, *Iflaviridae*), this research aims at elucidating the mode of transmission and tissue tropism of both iflavirus species. Firstly, linear DNA digestion by Plasmid-Safe-DNase proved the absence of circular dsDNA intermediates during the infection cycle of these viruses, unlike other RNA viruses that infect flies and mosquitoes. Next, the presence of the iflaviruses in all the host developmental stages was investigated by RT-qPCR. Both viruses were observed in all stages, albeit displaying different virus levels. In particular, while SeIV1 virus abundance was observed to be higher for the later life stages, the opposite was seen for SeIV2. Moreover, RT-qPCR proved the presence of these covert viruses in the insects' reproductive tissues (5th instar larvae and adults) and in other tissues, including fat body, gut and salivary glands. Interestingly, SeIV2, but not SeIV1, showed significantly higher abundance in adult testes compared to ovaries. However, both viruses appeared in high titres in gut tissues. These findings suggest both vertical and horizontal transmission. To further study possible vertical transmission, iflavirus-infected and uninfected adults were mated and their progeny were monitored for infection. Moreover, to investigate horizontal transmission, infected and uninfected larvae were allowed to share resources for 24hrs. We found that while both iflaviruses were mainly horizontally transmitted, vertical transmission also occurred. Furthermore, efficiency of SeIV1 vertical transmission was higher compared to SeIV2. To conclude, we showed that SeIV1 and SeIV2 use a mixed-mode transmission strategy, although differences between the two iflaviruses are observed. Further characterisation of such infections will lead to innovative pest-control techniques development.

Unveiling Covert Baculovirus Infection: Insights from Laboratory Trials on *Helicoverpa armigera* under Stress Conditions

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In integrated pest management, baculoviruses provide numerous benefits and enable a reduced use of synthetic chemical insecticides. Apart from directly causing mortality in treated larvae, baculoviruses exert additional effects on insect populations. In cases of sublethal infection, the virus can be vertically transmitted to the next generation. Recent studies have documented the presence of baculoviruses in Lepidoptera populations as covert infections, which can be activated by various stress factors. We conducted laboratory trials on *Helicoverpa armigera* using the active ingredient *Helicoverpa armigera* nucleopolyhedrovirus. The results provide insight into how these baculoviruses influence population development even in the generations following application.

MARTIGNONI AWARD

Effect of domestication on the repertoire of viruses and bacteria associated with the Mediterranean fruit fly, *Ceratitis capitata*

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The Mediterranean fruit fly or medfly (*Ceratitis capitata)* is an agricultural pest of a great variety of fruit crops. The sterile insect technique (SIT) is applied across the globe for the management of medflies. This technique relies on the release of sterile males that are mass-produced under laboratory-rearing conditions, which differ from the insect´s natural habitat. In this work, we focus on the effect of domestication, described as the transition from field to laboratory-rearing conditions, on the levels of RNA viruses and bacteria associated with medflies.
Thirteen RNA viruses have been described in the medfly, with medfly field populations presenting higher viral diversity than laboratory-reared populations. Our results confirmed the high diversity of RNA viruses in field-captured flies and revealed that domestication affected each RNA virus differently. Two RNA viruses maintained constant levels across twelve generations. Instead, domestication favored the replication of four RNA viruses, which increased their viral RNA levels over twelve generations and negatively affected three RNA viruses, for which viral RNA levels decreased. Additionally, our results revealed that medfly RNA viruses are a mixture of genotypes, which appeared at different proportions after the domestication process. The medfly bacteriome in field-captured medflies is dominated by bacteria of the Enterobacteriaceae order, in agreement with previous reports. Each genus of bacteria was differently affected by domestication. The proportion of *Klebsiella* decreased after a few generations of laboratory adaptation, while the proportion of *Moellerella*, *Citrobacter,* and *Pluribacter* increased by laboratory-rearing conditions.
Overall, this study reports the first insights into the influence of domestication on the repertoire of microorganisms associated with the medfly. Understanding these interactions will be crucial for maintaining the success of biological control methods in agricultural settings.
Keywords: RNA virus, covert infections, Bacteriome, Tephritidae, insect mass-rearing

Potential of a new *Glossina morsitans morsitans* cell line for isolation and propagation of tsetse fly viruses

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Tsetse flies of the genus *Glossina* are important vectors of salivarian trypanosomes in sub-Saharan Africa and are major constraints on livestock production, agricultural development and human health in the region. *Glossina* spp. are reported to harbour several insect-only viruses with potential application in tsetse fly control, including salivary gland hypertrophy virus (SGHV), Glossina morsitans morsitans iflavirus (GmmIV) and Glossina morsitans morsitans negevirus (GmmNegeV); to date, no *in vitro* culture systems have been reported for any of these viruses. We recently established a new tsetse fly cell line, GMA/LULS61, derived from tissues of adult female *Glossina morsitans morsitans*. To assess its possible use in isolation and propagation of tsetse fly viruses, we PCR-screened DNA and RNA extracted from GMA/LULS61 cells at passage 14 for, respectively, a specific sequence of *Glossina pallipides* SGHV, and the RNA-dependent RNA polymerase (RdRp) genes of GmmIV and GmmNegeV. The cell extracts were positive for both RdRp genes, indicating possible persistent infection with GmmIV and GmmNegeV, whereas the SGHV PCR failed to amplify any product, indicating absence of this virus. If further studies confirm presence of replication-competent GmmIV and GmmNegeV in the GMA/LULS61 cell line, this will represent the first long-term culture system for these two RNA viruses. Moreover, the cell line can be tested for ability to support replication of SGHV introduced from other tsetse fly species, thereby providing the first culture system for this DNA virus. In summary, the GMA/LULS61 cell line, available from the Tick Cell Biobank at the University of Liverpool, has potential for application in a variety of studies investigating the biology and control of *G. m. morsitans* and its associated pathogenic and symbiotic microorganisms.

Isolation of Iflaviruses of the testse fly *Glossina morsitans morsitans*

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Tsetse flies are a major threat to human and livestock health as they transmit Trypanosoma. Trypanosoma is a protozoan parasite causing sleeping sickness in humans (Human African Trypanosomiasis) and nagana in animals (Animal African Trypanosomiasis). Approximately 70 million people are at risk of contracting sleeping sickness and 4.5-billion-euro losses are accrued yearly in agricultural revenue due to its disease impact on livestock. Effective drug treatment for livestock does exist, but constant usage has led to drug-resistant Trypanosoma. The sterile insect technique (SIT) proved a more environmentally friendly and vector-specific control technique. To be successful with this technique, the mass-reared males need to outcompete the wild-type males. Therefore, our goal is to have healthy and competitive males. In this project, we investigate the influence of Glossina morsitans morsitans

iflavirus (GmmIV) and Glossina morsitans morsitans negevirus (GmmNegeV) on the health of the mass-reared tsetse fly. To study the effect of the different viruses individually, we developed different techniques to isolate the viruses. We are particularly interested to understand the effects on, iflavirus as it has been proven pathogenic in other insects. We isolated GmmIV from *Glossina morsitans morsitans* via passaging it three times via the cell lines *Spodoptera frugiperda* Sf9 and aedes *albopictus* C6/36. By injecting a purified GmmIV stock we will be able to study the immune response of the flies and see the effects this virus has on the tsetse.

CONTRIBUTED PAPERS **V-21-STU**

Novel RNA viruses of the tomato leafminer *Tuta absoluta*

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The tomato leafminer, Tuta absoluta, originating from South America, has become a widespread pest in tomato crops across 90 countries. Conventional pest management heavily relies on insecticides, prompting the search for chemical-free alternatives such as the use of entomopathogens. In this study, we have used meta-transcriptomics and bioinformatics approaches to obtain the RNA virome from multiple T. absoluta samples from different geographical locations and health status. Analysis of 16 different metatranscriptomes revealed 24 viral sequences, including seven putative insect-specific viruses (ISVs) classified into six groups of single-stranded RNA viruses. Additionally, four plant viruses and 13 fungal viruses were detected. Notably, none of the identified viruses were present across all studied samples and one virus belonging to the Iflaviridae family was exclusively detected in samples obtained from deceased larvae, suggesting potential for further investigation. Moreover, we have evaluated the prevalence of the discovered RNA viruses in transcriptomic datasets from T. absoluta populations worldwide. Surprisingly, only one ISV was identified across these populations, while Tomato mosaic virus (ToMV) shows widespread prevalence among T. absoluta populations globally. Our study marks the first characterization of the RNA virome of the tomato leafminer, providing valuable insights into its ecology and potential implications for pest management strategies.

CONTRIBUTED PAPERS **V-22-STU**

Re-emergence of homeostatic microbiome of mud crab *Scylla serrata* post-dysbiotic shift triggered by White Spot Syndrome Virus (WSSV) infection

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White spot syndrome virus (WSSV), the causative agent of white spot disease, remains a serious threat to crustacean aquaculture. Known to infect a wide range of crustaceans, host species exhibit varying susceptibility and mortality rates. The mud crab *Scylla serrata*, a high value aquaculture species across the Indo-West Pacific, is considered less susceptible and relatively resistant to the virus, making it a good model organism to investigate the natural mitigation strategies of a relatively WSSV-resistant host. Host-associated microbiomes, established to be important in maintaining overall host health are relatively understudied in the context of their potential role in mitigating host response to WSSV infections. This study used high-throughput sequencing of the full-length 16S rRNA gene to characterize the microbial community structure and dynamic change associated with different tissues of *S. serrata* in response to WSSV infection. Mud crabs were intramuscularly injected with WSSV inoculum (infected) or phosphate buffered saline (control) and monitored for 144 hours post injection (hpi). Tissue biopsies were collected at selected timepoints during infection: at onset (0, 12, 24-hpi), peak (48, 72-hpi) and drop (96, 120, 144-hpi) based on established viral load curves. Principal component analysis revealed a significant change in the microbial composition in the gut, gills, and hepatopancreas during the onset and early stage of infection. This suggests that WSSV infection is associated with a dysbiotic shift altering the community structure of the mud crab microbiome. However, despite the disruption of the healthy microbiome, mud crabs can regain their microbial community structure similar to the non-infected state at the drop stage of infection. This result suggests that mechanisms involved in the 'healthy' microbiome re-emergence likely contribute to mud crab resistance to the disease. Characterization of tissue-specific microbiome assemblages during WSSV infection provides valuable information to delineate the potential role of host-associated microbiome in host immunity.

CONTRIBUTED PAPERS **V-23-STU**

Comparative transcriptome analysis unveils varied host responses to sacbrood virus infection in *Apis cerana* and *Apis mellifera*

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SBV, affecting both *A. mellifera* (AmSBV-AM) and *A. cerana* (AcSBV-AC), primarily targets honey bee broods, leading to larval fatalities. Previous studies have demonstrated cross-infections of AcSBV-AC and AmSBV-AM in both species of honey bees and resulted in severe impact on *A. cerana*. This study attempted to elucidate the responses of *A. cerana* and *A. mellifera* to AcSBV-AC and AmSBV-AM infections. The result of transcriptome analysis revealed remarkable differences in the gene expression profiling between the two bee species after SBV infection. Gene Ontology (GO) analysis indicated a greater number of downregulation of developmental-related genes in the *A. cerana*/AcSBV-AC compared to those of *A. mellifera*/AmSBV-AM at 24 h.p.i. In addition, qRT-PCR was used to validate the expression of possible host-virus specificity genes in *A.cerana*/AcSBV-AC and *A.mellifera*/AmSBV-AM, revealing the potential of *RPA2* and *MUS81* to participate in SBV replication. Additionally, the downregulation of *Dpp* and *yellow-f* in the SBV infection was found to be reversely upregulated by SBV dsRNA treatment at 24 h.p.i.. Moreover, the upregulation of *Pyruva* in the SBV infection was found to be reversely downregulated by SBV dsRNA treatment at 48 h.p.i. This result suggested these genes may involve in host susceptibility and play a crucial role in virus-infected *A. cerana* larvae.

Exploring the virome of honey bee *(Apis mellifera*) and *Varroa* mite (*Varroa destructor*) in Taiwan through viral metagenomics

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The honey bee (*Apis mellifera*) is a crucial pollinator for many crops; however, it suffers from many biotic stresses, such as *Varroa* mites and viruses. Our study examined viral prevalence in honey bees and *Varroa destructor* from thirteen Taiwan apiaries. Subsequently, viral metagenomic analyses were conducted on samples from two apiaries using two high-throughput RNA sequencing strategies (rRNA depletion and poly A-based RNA-seq). From the results of metagenomic analysis, the prevalence of thirteen viruses was confirmed, including Deformed wing virus (DWV), Black queen cell virus (BQCV), and Kakugo virus (KV), Varroa destructor virus (VDV) in both honey bees and *Varroa* mites, while Sacbrood virus (SBV) and Lake sinai virus (four isolates of LSV) was only in honey bee samples. Apis rhabdovirus (ARV) was only found in *Varroa* mites. Our analysis also uncovered five newly recorded viral species in honey bee populations of Taiwan, such as Varroa orthomyxovirus (VOV), PNG bee virus, Darwin bee virus, Bundaberg bee virus, and Apis mellifera filamentous virus (AmFV). This is the first honey bee and *Varroa* mite virome analysis in Taiwan. Our data would be beneficial for establishing a honey bee colony health assessment index.

Comparison of bracovirus in incipient parasitoid species specialized on different hosts.

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Endoparasitoid wasps oviposit into and, through facilitation by symbiotic bracoviruses, undergo larval development within specific hosts. Recent studies have shown that parasitoid wasps often occur as in complexes of sister species, resulting from adaptations to specific hosts. We are studying the factors involved in the speciation of two populations of the wasp *Cotesia congregata* (CcC and MsT). CcC wasps parasitizes the caterpillar, *Ceratomia catalpa*, which specializes on the catalpa tree. MsT wasps parasitizes the caterpillar, *Manduca sexta,* which specializes on solanaceous plants, including tobacco. Differences in reproductive behavior and genetic differentiation (microsatellites and COI) demonstrate that these two populations are undergoing ecological speciation. The bracovirus, a nudivirus endogenized in the wasp genome and associated with tens of thousands of braconid wasps, is necessary for successful development of parasitoid larvae within the host. Bracovirus particles are produced in the wasp ovaries and injected into the host during oviposition. Then, particles infect parasitized host cells and express virulence genes that interfere with host immune defenses, physiology and development. These virulence genes are thought to contribute to host adaptation. We have obtained high quality assemblies of CcC and MsT genomes using long-read sequencing (PacBio) and precisely compared the sequences of bracovirus to identify differences in their content (virulence genes and mobile elements) that may be involved in host adaptation. We identified several events of gene loss, pseudogenization, tandem duplication and a different profile of mobile elements insertions.

Transcriptomic evidence for a conserved, nudiviral RNA polymerase in the parasitoid wasp, *Microplitis demolitor*, and the implications on *Bracovirus* gene discovery

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The braconid wasp, *Microplitis demolitor*, utilizes an endogenized beneficial virus, *Microplitis demolitor* bracovirus (MdBV), to combat the immune system of its larval-stage lepidopteran hosts so that the wasp progeny can safely develop. The MdBV genome is contained within the *M. demolitor* genome and replicates only in the wasp ovaries, while MdBV virulence only occurs in the tissues of wasp hosts such as Chrysodexis includens (the soybean looper). The MdBV replication genes include both early and late-transcribed viral genes, where some of the "early" genes encode a conserved, nudiviral RNA polymerase. In related, entomopathogenic baculoviruses, this RNA polymerase transcribes the "late" genes responsible for viral structure. Previous work with qPCR shows similar patterns in MdBV, but this study utilizes a global, transcriptomic analysis of gene expression following the RNAi-mediated knockdown of three nudiviral RNA polymerase subunits (lef-4, lef-8, and lef-9) to confirm the conservation of this role. After the viral RNA polymerase knockdown, 70% of "late" viral genes are significantly downregulated compared to control samples, while less than 0.1% of wasp genes show significant downregulation. Following this discovery, we predicted that additional viral genes could be identified based on their dependence on the nudiviral RNA polymerase. Since viral genes are traditionally identified via homology to known viruses, annotation of *Bracovirus* genes has been limited due to the fast evolution of endogenized virus gene sequences. To annotate any previously unknown genes in the MdBV genome, we used a bioinformatics pipeline to identify putatively-viral genes with the transcriptomic data generated in this study and successfully added 30 putatively-viral genes to our database. Thus, this work provides evidence for the conserved role of the ancestral, nudiviral RNA polymerase in MdBV gene transcription and demonstrates a novel way of identifying endogenized *Bracovirus* genes.

Suppressons: Ancient Nudivirus Virions Exapted to Form Parasitoid Organelles that Suppress Host Immunity

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Particles suppressing host immunity produced by certain female parasitoid wasps have been interpreted as virions of symbiotic viruses referred to as polydnaviruses (PDVs) for 50 years, despite lack of replication in lepidopteran hosts. In 2004, PDV researchers determined the virions produced by a braconid contained genes that suppress immunity, but no genes coding for viral structural proteins or replication. Later, it was determined genes for particle synthesis were derived from an ancient nudivirus integrated into braconid chromosomes. Genes for similar particles are integrated in ichneumonid parasitoid chromosomes. It was clear by 2004 that the virions of these symbiotic viruses were not virions, as they lacked viral genomes and do not replicate in lepidopteran hosts. Nevertheless, PDV researchers continue to use the symbiotic paradigm, still referring to the particles as virions. Doing so ignores alternative concepts suggesting ancient virus genomes in wasp chromosomes underwent exaptation, forming organelles that suppress innate immunity. Ironically, PDV researchers generated excellent evidence that favors an organelle paradigm over one for symbiotic viruses. The International Committee on Taxonomy of Viruses recently reclassified the parasitoid viruses as viriforms, genetic

elements that evolved from viruses, but have different functions. Indeed, other interpretations of molecular evidence demonstrate the particles function inside host target cells as organelles. The term suppressons, based on functions, is suggested to replace virions. The change in parasitoid larval ecology from ectoparasitism to endoparasitism likely drove the exaptation of ancient nudivirus genomes to synthesize suppressons.

CONTRIBUTED PAPERS **V-28**

Using AmE-711 Honey Bee Cells to Examine Virus - Fungicide Interactions at the Cell Level

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As pollinators of agricultural and natural ecosystems, honey bees (*Apis mellifera*) play an important role in maintaining our quality of life. However, these beneficial insects are frequently challenged by several known stressors that erode their health and productivity. Pesticides, mite parasites, pathogens, and nutritional deficiencies, often acting simultaneously, create disease conditions in hives that require intervention and the commitment of significant resources geared towards mitigating negative outcomes. Improving honey bee health is a goal for the beekeeping industry and calls for a comparative examination between healthy and diseased honey bees using cell-based, whole bee, and colony-level approaches that incorporate not only single, but more importantly, combinations of stressors. Cell-based studies are largely underdeveloped for honey bees; therefore, the aim of this study was to investigate the interactive effects of concurrent exposure to an agriculturally-relevant fungicide, chlorothalonil, and infection with two common honey bee viruses. Deformed wing virus (DWV), which is a persistent infection of AmE-711, and Acute bee paralysis virus (ABPV) were chosen as model honey bee viruses. Cell viability and mitochondrial membrane potential assays, relative quantification of immune and oxidative stress gene expression, and cell morphological analysis were conducted to assess the response of AmE-711 honey bee cells to fungicide exposure and viral infection. Preliminary findings show changes mainly in response to single stressors and highlight the need for additional research using AmE-711 as a platform for developing models of interacting stressors that are relevant to honey bee health.

CONTRIBUTED PAPERS **V-29**

Inhibition of medically important mosquito-borne viruses by the Insect-Specific Flavivirus Binjari across acute and persistent states in mosquito cells

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Annually more than 400 million people are infected by mosquito-transmitted viruses such as West Nile virus (WNV), Zika virus (ZIKV) and chikungunya virus (CHIKV). Transmission of these viruses by mosquito vectors can be suppressed by prior infection of the mosquito with insect-specific viruses (ISV), suggesting a means to reduce disease transmission. However, these interactions are generally studied during an acute ISV infection in *Aedes albopictus* C6/36 mosquito cells. These cells are defective in their main antiviral response (siRNA), and as ISVs persistently infect their mosquito host, acute infections in C6/36 cells are likely not a suitable model for these studies.
To study mosquito immunity and resistance to secondary mosquito-borne virus infections in a more representative model system we created C6/36 and *Aedes aegypti* Aag2 cells that are persistently infected with the insect-specific flavivirus Binjari virus (BINJV). In our experiments, acute infections with BINJV caused strong cytopathic effects (CPE) in C6/36 cells, whereas CPE was milder in Aag2 cells with intact antiviral responses. Serial passaging of BINJV infected cells resulted in persistently infected cell lines that maintained low-level BINJV replication and had a healthy appearance. Replication of WNV, ZIKV and CHIKV was inhibited by prior infection with BINJV, however this inhibition was more pronounced during acute BINJV infection compared to persistent infection and stronger in the C6/36 cells compared to Aag2. When BINJV was genetically modified to contain a part of the ZIKV sequence, Aag2 cells were effectively protected from secondary ZIKV infection.
Together, this research shows that prior infection with an ISV can reduce infection of a secondary mosquito-borne virus. However, without sequence homology, inhibition of a secondary infection by persistently replicating BINJV is limited compared to acute and more cytopathic primary infections. This suggests that during acute infections multiple distinct mechanisms contribute to superinfection exclusion.

CONTRIBUTED PAPERS **V-30**

***Aedes albopictus* response to Cell fusing Agent virus infection in relation to temperature.**

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Aedes albopictus, is the primary arboviral vector in temperate areas of the world. Besides arboviruses, *Ae. albopictus* can support replication of numerous Insect-Specific Viruses (ISVs). ISVs are gaining interests in the scientific community for their potential applications as biological control agents for the control of arboviral infections inside the vector. Being ectotherms, mosquitoes bionomics and control need to be considered in relation to current global warming. Here, we investigated how exposure to 32 °C for one generation (warm acclimated mosquitoes) *vs* ten generations (warm-evolved mosquitoes) affects the response of mosquitoes to the infection by the ISV Cell-Fusing Agent Virus (CFAV) in comparison to standard reared mosquitoes (28 °C). We specifically tested whether resistance, tolerance or the reproductive capacity of each mosquito group changes after CFAV infection. We found that warm-acclimated mosquitoes are more refractory to CFAV infection, but they suffer a fitness cost in terms of reduced fecundity with respect to warm-evolved and standard reared mosquitoes. On the contrary, warm-evolved mosquitoes reach higher levels of CFAV loads than warm-acclimated and standard reared mosquitoes but show no fitness costs. We conclude that thermal stress alters how mosquito react to CFAV infection depending on the length of the thermal challenge.

Efficacy of sixteen nucleopolyhedrovirus isolates against *Helicoverpa armigera, Spodoptera litura*, and *Trichoplusia ni* in Sri Lanka

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Numerous nucleopolyhedrovirus (NPV) strains discovered worldwide have been used as biopesticides extensively to manage crop pests. Different isolates of NPVs have exhibited variations in their genetics, pathogenicity, and field efficacy. The efficacy of 16 NPV isolates identified from *Helicoverpa* species was evaluated against *Helicoverpa armigera, Spodoptera litura,* and *Trichoplusia ni* collected in Sri Lanka. In laboratory bioassays, LC_{50} estimates varied over an approximately 2-fold range, with five NPV isolates (Biotrol Texas USA, Gemstar Lot#35022, 3154 Russia, 1072 China, and 1623 India) demonstrating higher virulence than other NPV isolates against *H. armigera* second instar larvae. These five isolates were selected and evaluated for their capacity to control *H. armigera* in field trials. Isolates 1072 China and 1623 India expressed the highest efficacy against *H. armigera* on tomato plants under field conditions. The isolates exhibited little to no virulence against larvae of *S. litura* in laboratory bioassays while inoculation with 3154 Russia resulted in 100 % mortality in *T. ni* 2nd instar larvae within 10 days. The negative traits of NPV bio-pesticides such as slow speed of kill and limited host range can be overcome by selecting the most suitable isolate. The application of NPV biopesticides at the initial stage of pest infestation, under suitable environmental conditions, and multiple times can ensure higher pest control efficacy.

The expression of CrpeNPV gp37 as a formulation additive for improved infectivity with CrleGV against *Thaumatotibia leucotreta*.

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The False codling moth, *Thaumatotibia leucotreta*, is a significant pest of several agricultural crops in South Africa, such as citrus and macadamia. Two baculoviruses, Cryptophlebia leucotreta granulovirus (CrleGV) and Cryptophlebia peltastica nucleopolyhedrovirus (CrpeNPV), have been developed into commercial biopesticides for the control of this pest. Previous research evaluating dual infections with CrleGV and CrpeNPV against *T. leucotreta* resulted in a decreased lethal concentration 50 (LC_{50}), however, increased lethal time 50 (LT_{50}) was observed. A previous study reported a significant decrease in the LC of Spodoptera exigua NPV and Autographa californica NPV following the addition of the protein gp37 from Cydia pomonella granulovirus during infection. The *gp37* gene has been identified in CrpeNPV, but not in CrleGV, and may be responsible for the decreased LC observed during dual infections. The aim of this study was to establish a bacterial expression system for the production of gp37 to enable future evaluation of this protein. A recombinant plasmid containing the CrpeNPV *gp37* gene fused to a His-SUMO-tag was constructed to evaluate expression in Rosetta BL21 (DE3) *E. coli* cells. A time course induction study was performed at 37, 25, and 18 ℃. Optimal expression was achieved at 18 ℃, however, subsequent attempts to improve solubility and purification of the protein have encountered difficulties. A second recombinant plasmid containing the CrpeNPV *gp37* gene with a partial 5' truncation, removing a hydrophobic domain, was constructed in an attempt to improve solubility. Expression was conducted at 18 ℃ in JM109 *E. coli* cells, with two IPTG concentrations evaluated. A decreased IPTG concentration showed increased soluble protein expression, a necessary step for downstream purification and quantification. Further optimisation of induction parameters, improved protein purification protocols, and evaluation of gp37 as an additive for CrleGV against *T. leucotreta* are underway.

Carbon quantum dot nanoparticles increase the efficacy of Spodoptera littoralis nucleopolyhedrovirus suspoemulsion

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Carbon quantum dots (CQDs) are carbon nanoparticles with a size less than 10 nm and surface coating. They are currently used for bioimaging due to their fluorescence features as well as drug delivery in biomedicine. Recent studies revealed that they also have a potential for nanoparticle-based pesticide formulations with improved stability and slow release of active insecticidal molecules that lead to longer duration of pest control. However, their potential in biopesticide formulation is poorly known. In the current study, we investigated the insecticidal potential of CQDs in combination with Spodoptera littoralis nucleopolyhedrovirus (SpliNPV) (belonging to the species *Alphabaculovirus splittoralis*) against second-instar larvae of *Spodoptera littoralis,* both under laboratory and greenhouse conditions. Individual application of CQD led to death in the larvae; however, their combined use with SpliNPV led to increased mortality and reduced lethal time under laboratory conditions. Under greenhouse conditions, incorporation of CQD into a suspoemulsion bioformulation enhanced the virus's efficacy, resulting in 100% mortality on eggplant and 93.51% on pepper plants. To understand the potential mode of action of CQDs, we also investigated the expression of select defensive midgut genes in response to CQD *per os* delivery, which revealed significant and specific up-regulation. Overall, the current study underscores the potential of CQD as a novel tool in integrated pest management and highlights the importance of understanding the synergistic interactions between nanoparticles and biocontrol agents for enhanced pest control efficacy.

A CRM1-Dependent Nuclear Export Signal in Autographa californica Multiple Nucleopolyhedrovirus Ac93 Is Important for the Formation of Intranuclear Microvesicles

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Autographa californica multiple nucleopolyhedrovirus (AcMNPV) Ac93 is highly conserved in all sequenced baculovirus genomes, and it plays important roles in both the nuclear egress of nucleocapsids and the formation of intranuclear microvesicles. In this study, we characterized a cellular CRM1-dependent nuclear export signal (NES) of AcMNPV Ac93. Bioinformatic analysis revealed that AcMNPV Ac93 may contain an NES at amino acids 115-125. Green fluorescent protein (GFP) fused to the NES (GFP:NES) of AcMNPV Ac93, localized to the cytoplasm of transfected cells. Multiple point mutation analysis demonstrated that the

NES is important for the nuclear export of GFP:NES. Bimolecular fluorescence complementation experiments and co-immunoprecipitation assays confirmed that Ac93 interacts with *Spodoptera frugiperda* CRM1 (SfCRM1). However, AcMNPV Ac34 inhibits cellular CRM1-dependent nuclear export of GFP:NES. To determine whether the NES in AcMNPV Ac93 is important for the formation of intranuclear microvesicles, an *ac93*-null AcMNPV bacmid was constructed; the wild-type and NES-mutated Ac93 were reinserted into the *ac93*-null AcMNPV bacmid. Immunofluorescence analysis showed that Ac93 and SfCRM1 were predominantly colocalized at intranuclear microvesicles in infected cells, while the construct containing point mutation at residues 123 and 125 of Ac93 resulted in a defect in budded virus production and the abolishment of intranuclear microvesicles. Together, these data demonstrate that Ac93 contains a functional NES, which is required for production of progeny virus and the formation of intranuclear microvesicles.

CONTRIBUTED PAPERS **V-35**

Expression of viral antiapoptotic genes during the development of SfNPV-Ar baculovirus infection in its host *Spodoptera frugiperda*

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Programmed cell death or apoptosis is the combination of cellular processes whose purpose is to eliminate the first infected cells, which have irregular functions or effects on the cell cycle. During the development of baculovirus infection, in the cells of susceptible insects, the activation of this process occurs when the viral DNA replicates and when certain genes of the viruses themselves are expressed, however, baculoviruses have gene families specialized in inhibit apoptosis. In the present study, the presence of antiapoptotic genes in the genome of the SfNPV-Ar viral strain was analyzed, as well as their expression at different times during the development of the primary infection in vivo in its host *S. frugiperda*. This viral strain possesses the antiapoptotic genes *iap-2, iap-3, p45, p49*, and *ring*. Subsequently, using the ss cDNA synthesized from the RNA of the infected tissue at 6, 12 and 24 hpi, specific oligonucleotides designed for each gene and PCR, it was determined that the baculovirus strain in the intestine of S. frugiperda expressed the gene *iap-2* at 24 hpi, the *iap-3* gene at 12 and 24 hpi, the *p45* gene at 24 hpi, the *p49* gene at 12 and 24 hpi, while the *ring* gene was expressed throughout the infection process. at 6, 12 and 24 hpi. The present study demonstrated the complex interaction that exists between the viral strain and its host, by determining both the presence of various antiapoptotic genes in the virus genome, as well as the time in which these are expressed, which responds to the needs of viral defense by the insect, demonstrating that the SfNPV-Ar viral strain is extremely efficient for the control of *S. frugiperda*.

PLENARY SYMPOSIUM **8-1**

The Sterile Insect Technique: its application and challenges

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The sterile insect technique (SIT) is an environment-friendly method of pest control that integrates well into area-wide integrated pest management (AW-IPM) programmes. For several major insect pests, the SIT is being applied. This technology, using radiation to sterilize insects, was first developed in the USA, and is currently applied on six continents. For almost six decades it has been a major subject for research and development in the Joint FAO/IAEA Programme on Nuclear Techniques in Food and Agriculture, involving both research and the transfer of this technology to Member States so that they can benefit from improved plant, animal and human health, cleaner environments, increased production of plants and animals in agricultural systems, and accelerated economic development. The socio-economic impacts of AW-IPM programmes that integrate the SIT have confirmed the usefulness of this technology. As strategic options they include, “suppression”, “eradication”, “containment”, and “prevention”, in which the SIT can be deployed as part of AW-IPM programmes, are defined and described in relation to the contexts in which they are applied against nonnative invasive or naturally occurring major insect pests. Advantages and disadvantages of these strategic options are analysed, and examples of successful programmes provided. Considerations that affect decision making in relation to the selection of a strategic option, such as. pest biology, whether the pest is a disease vector or agricultural pest, the pest’s status in the target area, and the target market for the produced crop or livestock commodities, are reviewed and discussed in terms of a phased conditional approach to programme planning, preparation, and implementation. The choice of a strategic option needs to be assessed carefully, and preparations should be supported by considerable baseline data, technical and economic feasibility assessments, and detailed planning. In addition, to set the scene for the conference, the latest developments in the technology: managing pathogens in insect mass-rearing, using symbionts and modern molecular technologies in support of the SIT, applying post-factory nutritional, hormonal, and sociochemical treatments, applying the SIT to eradicate outbreaks of invasive pests, and using the SIT against pest of agricultural, veterinary and medical importance.

PLENARY SYMPOSIUM **8-2**

The application of culturing, DNA and RNA community sequencing in identifying and developing diagnostic assays for microbial pathogens in *Anastrepha ludens* mass-rearing facilities

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The Mexican fruit fly, *Anastrepha ludens*, is a major agricultural pest of citrus that is capable of using over 40 plant species of fruits and vegetables as hosts. Native to Mexico, this pest is a constant threat to U.S. agriculture because of its proximity to citrus production in southern Texas and California. Sterile Insect Technique is an environmentally friendly and effective tool for the management and eradication of the Mexican Fruit Fly. In Texas, the release of SIT flies is a crucial part of safeguarding agriculture. SIT requires millions of sterile male flies to be released each week that come from mass-rearing facilities. Microbial infections can cause a decrease in sterile fly production. Initially media-based culture techniques were used to identify bacteria and fungi found in the insect in the lab-reared and mass-reared facilities, followed by small cup pathogenicity tests to determine the phenotypic consequences of the isolates. Community based sequencing of 16s rRNA for bacteria and archaea and COI for the major phyla of fungi can identify microbes without the biases of culturing. The most recent advancement in sequencing is total RNA sequencing of the prokaryotic and eukaryotic microbial communities. In addition to identifying all microbes in one sample without multiple gene libraries, the community transcriptome also indicates when and where the microbes are active and the possible RNA and protein activity of the microbes. The three techniques of culturing, gene sequencing and transcriptome sequencing have identified multiple pathogens such as *Morganella*, *Providencia,* and *Zygosaccharomyces* for pathogenicity and possible pathogens such as *Serratia, Klebsiella, Microsporidia* and *Lecanicillium*.

PLENARY SYMPOSIUM **8-3**

Management of viruses in insect mass rearing facilities for the sterile insect technique: *Glossina pallidipes* salivary gland hypertrophy virus (GpSGHV) as an example

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Effective management of insect viruses in insect mass-rearing facilities is fundamental for a successful implementation of the sterile insect technique (SIT), a component of an area-wide integrated pest management programme. The control of insect viral infections represents the main challenge in colony establishment and stability, as they reduce the insect performance like the mating and flight ability, thus affecting the sustainable production of high-quality males required for SIT. There is a broad range of insect pathologies, including those caused by pathogenic bacteria and fungi. Here, only the viral pathogens affecting the mass-reared insects such as tsetse flies and mosquitoes will be discussed, particularly the successful management of the *Glosinavirus* in tsetse colonies, and the risks of infection of pathogenic viruses and vector-borne diseases in mass-reared mosquitoes. Tsetse flies are naturally infected by *Glossina pallidipes* salivary gland hypertrophy virus (GpSGHV), a virus whose infection is characterized by salivary gland hypertrophy syndrome (SGH), leading to reproductive dysfunction of infected flies and colony collapse. The development of GpSGHV management strategies relied on understanding the modes of transmission of the virus, tsetse biology and the rearing system in tsetse colonies. These include a clean blood feeding system that aims to reduce horizontal virus transmission and the administration of the antiviral drug valacyclovir that targets the GpSGHV DNA polymerase to inhibit virus replication. The application of this combined GpSGHV management strategy, has led to the elimination of SGH, and has ultimately resulted in complete elimination of virus infections in tsetse colonies at the Insect Pest Control Laboratory in Seibersdorf. This successful example of a virus management system in tsetse colonies can be used as a model for developing virus management strategies in insect factories for SIT application or even in insect food companies.

PLENARY SYMPOSIUM **8-4**

Challenges and solutions to viral contamination in mass rearing of codling moth and false codling moth for the sterile insect technique

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The sterile insect technique (SIT) is the most suitable method for area-wide suppression of insect pest populations. Large numbers of laboratory-reared insects are irradiated to sterilize them. Partially or fully sterilised insects are then mass released at a determined minimum overflooding ratio to their wild counterparts. This leads to a greater probability of wild insects mating with their sterile counterparts than with other wild insects, inducing population suppression. SIT requires rearing of the pest insect in question on a massive scale. Codling moth has been mass reared for an SIT programme in British Columbia, Canada, since 1992, and was mass reared in South Africa for a similar programme, which only lasted for four years. A false codling moth SIT programme has been running in South Africa since 2007. Virus contamination can be seriously problematic in rearing systems for both species, undermining the ability of the programme to produce the requisite number of insects with the required fitness and competitiveness. Various stress factors and injudicious management practices have been identified that can exacerbate virus outbreaks. These include larval rearing in open trays, overcrowding, deficient temperature and humidity settings, inadequate air filtration, water contamination and deficient hygiene protocols. This can result not only in culture crashes, in the case of overt infections, but also reduced fitness and fecundity in the case of covert infections, affecting both laboratory rearing and field performance of released moths. There could also be more than one species of virus responsible. A multiplex PCR technique and genome specific primers have been developed to not only identify the occurrence of virus contamination, but determine which species is responsible. Numerous practices have also been adopted to reduce and resolve these virus contamination issues.

MICROSPORIDIA DIVISION SYMPOSIUM **9-1**

Microsporidian Taxonomy: Things to Tie Up

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Microsporidian taxonomy has been in flux over the last century, first led and informed by morphological-based systematics and later incorporating molecular detail. Over time, many microsporidian genera have been amended/updated due to greater insight into their evolutionary relatedness and ecology. Specific cases include recent rearrangements and re-naming of some members of the Nosema and Thelohania, as well as the development of novel higher classification systems. I'll aim to summarise recent changes, their validity and ongoing debates, as well as where we need to explore novelty in microsporidian taxonomy.

MICROSPORIDIA DIVISION SYMPOSIUM **9-2**

Effects of *Vairimorpha* (*Nosema*) *ceranae* and *Lotmaria passim* infections on honey bee behaviour and physiology

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Vairimorpha (*Nosema*) *ceranae* and *Lotmaria passim* are two common, globally encountered digestive tract parasites of the honey bee (*Apis mellifera* L.). Though these parasites have previously been associated with colony losses, little is known about how they affect bee behaviour and physiology, or how they may be contributing to colony losses. Here, we will discuss the results of a field experiment where we used locally-obtained isolates of *N. ceranae* and *L. passim* to illustrate that these parasites, particularly in combination, are capable of altering bee physiology leading to changes in foraging behaviour. Changes in bee physiology were measured by quantifying vitellogenin expression in experimental bees at three separate time points. We observed experimental bees daily for the duration of the experiment to quantify changes in foraging behaviour. At the first instance of foraging, honey bees infected with both *N. ceranae* + *L. passim* had the lowest vitellogenin expression of all four treatments examined, and had a significantly younger average foraging age (0.6 days) compared to uninfected bees. Trends in parasite density and foraging effort from this experiment will also be presented. The changes in physiology and behaviour observed during this experiment have the potential to result in smaller, less productive colonies, reduced income for beekeepers, and ultimately, decreased colony survival. We will also discuss the

results of a cage level gene expression experiment that will provide information regarding parasite interactions and tissue preferences.

MICROSPORIDIA DIVISION SYMPOSIUM **9-3**

Microsporidian Genomes: Tales of polyploidy, rearrangements, and recombination

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Microsporidia are single-celled, spore-forming, obligately intracellular parasites with tremendous public health and economic importance, which infect a huge range of metazoan and protozoan hosts. The first microsporidian to be described was Nosema bombycis, the agent of a disease known as “pébrine” in farmed silkworms (Bombyx mori, Lepidoptera), which had caused huge collapses in the silk industry across the globe in the late 1800s. Since then, microsporidia have been identified as parasites of humans, as a causative agents of beehive collapses, and as emerging parasites in fisheries. Recently however, the microsporidians Vavraia culicis and “Microsporidia MB” were shown to be associated with a reduction in Plasmodium transmission in infected Anopheles, suggesting that microsporidia may represent a potential route to malaria control. Despite this, few microsporidian species have had their whole genomes sequenced, with much of their biology and pathogenicity remaining mysterious. In my PhD, I have endeavoured to generate as many novel microsporidian whole genomes as possible, relying on both my independent sampling of arthropods around the UK, and the vast diversity of organisms sequenced by the Darwin Tree of Life. Here, I present upwards of 25 high-quality microsporidian genomes from single hosts, in contrast to other publicly-available assemblies which have relied on cultured material or multiple pooled individual hosts. Using this data, we show that polyploidy is widespread in Microsporidia, and that the group possesses a unique genome architecture, rife with structural rearrangements (both between species and within the haplotypes of some polyploid genomes). Using phased microsporidian genomes we’re also able to reconstruct the arrangement of the haplotypes within a polyploid microsporidian and their interactions, illuminating unknown aspects of sexual reproduction in the group.

MICROSPORIDIA DIVISION SYMPOSIUM **9-4**

The molecular mechanisms underlying the vertical transmission of *Nosema bombycis* in silkworms provide an opportunity for molecular breeding

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Microsporidian *Nosema bombycis* is a typical microsporidium that infects multiple lepidopteran insects via fecal-oral and transovarial transmission (TOT); however, the underlying TOT processes and mechanisms remain unknown. Recently, we characterized the TOT process and identified key factors enabling *N. bombycis* to invade the ovariole and oocyte of silkworm *Bombyx mori*. We found that the parasites commenced with TOT at the early pupal stage when the ovarioles penetrated ovary wall and exposed to the hemolymph. Subsequently, the parasites in hemolymph firstly infiltrated the ovariole sheath, from where they invaded the oocyte via two routes: (I) infecting follicular cells, thereby penetrating oocytes after proliferation, and (II) infecting nurse cells, thus entering oocytes following replication. In follicle and nurse cells, the parasites restructured and built large vacuoles to deliver themselves into the oocyte. In the hemolymph, the parasites were coated with vitellogenin (BmVg), apolipophorin, 30 kDa protein10 (Bm30K10), transferrin, and storage protein, most of which have been reported to be transported into the oocyte. Suppressing expressions of these proteins and blocking their bindings with pathogens led to a significant decrease in pathogen loads within ovarioles. Among these proteins, BmVg was found to be present on the parasite surface within all ovariole cells. Additionally, Bm30K10 was found to bind with parasites inside nurse cells and oocytes. Both RNAi BmVg expression and block of its binding resulted in decrease of infections in all ovariole cells, while disruption of Bm30K10 suppressed pathogen load specifically within nurse cells and oocytes. These findings suggest that both BmVg and Bm30K10 play crucial roles in the TOT. Furthermore, our study demonstrated that all host proteins interacted with multiple spore wall proteins (SWPs). Moreover, we identified specific amino acid residues within BmVg that mediate its binding with SWPs, providing potential targets for blocking TOT of *N. bombycis* using genetic editing technologies.

VIRUSES SYMPOSIUM **10-1**

The diversity and impact of endogenous viral elements in insects

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Endogenous viral elements (EVEs) are pieces of viral genomes integrated into the genome of their hosts. Here I will provide an overview of the currently known diversity of EVEs in insect genomes, of the role of transposable elements in viral endogenization, as well as of the impact of EVEs on insect biology and evolution. Such EVEs have so far been annotated in at least eight insect orders and can be assigned to at least three families of large double-stranded (ds) DNA viruses, at least 22 families of RNA viruses, and three families of single-stranded DNA viruses. The study of these EVEs has already produced important insights into insect-virus interactions, including the discovery of a new form of adaptive antiviral immunity. Insect EVE diversity will continue to increase as new insect genomes and exogenous viruses are sequenced, which will continue to make paleovirology a vibrant research field in this group of animals in the years to come.

VIRUSES DIVISION SYMPOSIUM **10-2**

Filamentoviridae, an ancient family of DNA viruses influencing both the short- and long-term evolution in host-parasitoid systems

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Hymenopteran parasitoids are often associated with heritable viruses, some of which have a profound effect on their phenotype. However, their diversity is largely unknown. In this talk, I will show how we discovered a putatively large, ancient and diversified family of dsDNA viruses associated with hymenopteran parasitoids. The initial discovery was facilitated by the unexpected behavioural effect of one of its members on female wasps. I will therefore briefly describe the behavioural change induced by the virus and its potential consequences for the functioning

of the host-parasitoid community. Exploration of the diversity of this viral family suggests that they have specialized on Hymenoptera with parasitoid lifestyle since their early diversification. I will show how some lineages of this virus family have integrated into the chromosomes of some wasp clades, sometimes allowing important genetic innovations in these clades. Finally, endogenized versions of viral genes were also detected in a few non-hymenopteran insects, consistent with the possibility that they were linked to infected Hymenoptera through host-parasitoid relationships. We propose to call these viruses Filamentoviridae due to their common filamentous morphology. We propose that they constitute the fifth family in the *Naldaviricetes* class.

VIRUSES DIVISION SYMPOSIUM **10-3**

Endogenous Viral Elements in arboviral vectors: from discovery to functions

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Despite being overshadowed by the higher prevalence and more understood horizonal gene transfer among prokaryotes, the transfer of genetic material from DNA viruses and retroviruses to eukaryotic cells is a well-recognized phenomenon. Recent discoveries expand the range of viral types that can transfer genetic material to their hosts to non-retroviral RNA viruses. Endogenous Viral Elements (EVEs) from this type of viruses are called non-retroviral (nr)EVEs. Through a bioinformatic analyses of 22 mosquito genomes, we showed that the number of nrEVEs is disproportionally higher in arboviral vectors, such as *Aedes aegyti* and *Aedes albopictus*, with respect to vectors of protozoan parasites. We further showed that, in *Aedes* spp. genomes, nrEVEs comprise fragmented sequences, which are statistically-significantly ennriched in piRNA clusters, in association with LTR retrotransposons and encode for piRNAs. Additionally, some nrEVEs can improve tolerance to cognate viral infections in a tissue-specific manner. We also developed bioinformatic tools to characterise novel EVEs in the genome of wild-caught mosquitoes and showed that the patter of EVEs is variable across geographic populations, with novel integrations being rare, but continuous events, which are not correlated with the mosquito virome. Our results support the conclusion that the abundance of nrEVEs in *Aedes* spp. do not depend on viral exposure but seems to correlate with the landscape of transposable elements and the function of the piRNA pathway in these mosquitoes. More recently, we are focusing our attention to nrEVEs which are outside piRNA cluster, encompass complete viral open reading frame and are transcribed, as these characteristics suggest exaptation.

VIRUSES DIVISION SYMPOSIUM **10-4**

Endogenized nudiviruses in parasitic wasps and their fate in caterpillar hosts

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Interactions between organisms shape eco- and agrosystems. Parasitoid wasps play an important role in environments by regulating populations of their insect hosts which can be pests of plants. Among certain parasitoid wasps, remarkable parasitic strategies, involving the endogenization of viruses such as nudiviruses have evolved. Indeed, multiple events of nudivirus domestication have been described in the genomes of certain Braconidae and Campopleginae wasps. Each event taking a different evolutionary trajectory and leading to different virulence strategies involving the production of non-replicative viruses or virus-like-particles, essential for wasp parasitism success. The fate of non-replicative viruses in the insect hosts in which they are injected is also fascinating, as these viruses have an impressive capacity to integrate in all tissues of parasitized hosts. They have in consequence been mediators of horizontal-gene-transfers between different insect species, the scale and consequences of which are only starting to be evaluated both in natural ecosystems and in the context of biological control in agriculture.

DBI DIVISION SYMPOSIUM **11-1**

Sex ratio distorters and the evolution of host sex determination mechanisms

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In animals, sex is generally determined by genetic factors carried by sex chromosomes. Sex chromosomes are remarkably variable in origin and they can differ even between closely related species, indicating that transitions occur frequently and independently in different groups of organisms. However, the evolutionary causes underlying sex chromosome turnovers are poorly known. I will present results supporting that selfish genetic elements distorting host sex ratio can be powerful agents of transitions between sex determination mechanisms, as exemplified by feminizing *Wolbachia* bacterial endosymbionts in terrestrial isopods.
In the common pillbug *Armadillidium vulgare* (Crustacea, Isopoda), chromosomal sex determination follows female heterogamety (ZZ males and ZW females). In addition, many *A. vulgare* populations harbor maternally-inherited *Wolbachia* bacterial endosymbionts which can convert genetic males into phenotypic females, leading to populations with female-biased sex ratios. This bias selects against the W sex chromosome in lines infected by *Wolbachia*, such that all individuals are ZZ genetic males. Therefore, sex is only determined by the inheritance of *Wolbachia* by the *A. vulgare* individual, thereby leading to a shift from chromosomal to cytoplasmic sex determination. Surprisingly, some *A. vulgare* lines exhibit biased sex ratios despite the lack of *Wolbachia*. This bias is induced by the *f*-element, which we identified as a large piece of the *Wolbachia* genome recently transferred to the *A. vulgare* nuclear genome. The *f*-element is another sex-determining locus in pillbugs and the chromosome carrying the insert is a new feminizing chromosome.
Overall, our results indicate that sex ratio distorters can be powerful sources of evolutionary novelty for fundamental biological processes in animals, such as sex determination.

DBI DIVISION SYMPOSIUM **11-2**

A tale of two endosymbionts that affect host fitness and sex allocation via egg-size provisioning in a haplodiploid insect species

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Many arthropods are associated with maternally inherited endosymbiotic bacteria that affect reproduction. A common effect is cytoplasmic incompatibility (CI) between infected males and uninfected females leading to in embryonic mortality of fertilized eggs; reciprocal matings are compatible. CI provides a reproductive advantage to infected females, leading to endosymbiont spread. In contrast to diplodiploids, females of haplodiploids experiencing CI still have male offspring developing from unfertilized eggs, posing a challenge for endosymbiont invasion. Recently, another endosymbiont effect has been recognized in the modification of sex allocation. This was expected due to the maternal inheritance, yet underlying mechanisms remained unknown. We tested how CI-inducing endosymbionts *Cardinium* and *Wolbachia* interact to increase female production in naturally coinfected Kelly's citrus thrips, an Australian-native pest invasive in other parts of the world. This species has an egg size-dependent fertilization mechanism, and sex allocation depends on egg provisioning and maternal condition. *Cardinium* augments female production by increasing maternal fitness and egg size, thereby boosting fertilization rate and offspring fitness. *Wolbachia*, in contrast, reduces the beneficial *Cardinium* effects. Furthermore, *Cardinium* alters female mating behaviour in that *Cardinium*-infected females recognize *Wolbachia* infections in males to avoid CI costs – potentially an additional barrier to *Wolbachia* invasion. Global field surveys show that *Cardinium* is fixed throughout the host's distribution whereas coinfections with *Wolbachia* are restricted to eastern Australia where they occur at high prevalence. The *Cardinium*-*Wolbachia* coinfection is linked to particular mitochondrial haplotypes and lower haplotype diversity, suggesting that *Wolbachia* more recently infected this host already infected with *Cardinium*. Our findings demonstrate different invasion strategies and antagonistic effects of endosymbionts on host fitness, sex allocation and sexual selection.

DBI DIVISION SYMPOSIUM **11-3**

Invasion of the body snatchers: the role of parasite introduction in host distribution and response to salinity in invaded estuaries

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In dynamic systems, organisms are faced with variable forces that may impose selective trade-offs. Salinity is a major physical driver of estuarine diversity, while parasites are key biotic forces shaping host distribution and demography. We tested for trade-offs between low-salinity stress and parasitism in a castrating parasite and host crab, performing field surveys every 6-8wks over 3yrs along salinity gradients to determine factors influencing parasite prevalence, host abundance, infection probability, and taxa diversity. We examined demographic data from ~12,000 crabs, and analyzed temperature, salinity, and taxa data from 20 seasonal sampling events. Further, a lab experiment investigated signatures of low-salinity stress on host response (time-to-right and gene expression). We found salinity and temperature significantly affected parasite prevalence, with sites <10 PSU lacking infection, and populations in moderate salinities at warmer temperatures attaining prevalence as high as 60%. An individual's infection probability was driven by salinity, host size, and season, and host abundance was negatively associated with parasite prevalence. Gene expression was plastic to acclimation salinity, but several osmoregulatory and immune-related genes demonstrated source-dependent salinity response. We identified a salinity-associated genetic marker, suggesting possible selection on standing variation. Our study demonstrates how selective trade-offs in naturally dynamic systems can profoundly shape host evolutionary ecology.

DBI SYMPOSIUM **11-4**

Pathological dynamics of the sexually transmitted betanudivirus Heliothis zea nudivirus 1

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Nudiviruses are double-stranded DNA viruses that infect insects and crustaceans. Unlike most nudiviruses, which are orally transmitted, Helicoverpa zea nudivirus 2 (genus *Betanudivirus)* is sexually transmitted and causes malformations in the reproductive tissue of its lepidopteran hosts. Given the scarce pathological understanding of sexually transmitted nudiviruses, particularly at the molecular level, our study aimed to uncover the pathological mechanisms of the related Heliothis zea nudivirus 1 (HzNV-1) in the ovaries-derived (*Helicoverpa zea*) HzAM1 cell line.
Utilizing an established cell culture and infection system, we studied HzNV-1's cell entry mechanisms via electron microscopy and an inhibitory assay, and pinpointed the onset of viral DNA replication using quantitative PCR (qPCR). Furthermore, we harvested total RNA from infected cells at 3, 6, 9, 12, and 24 hours post-infection (hpi) and examined transcriptional changes in viral and host gene expression using RNAseq.
We observed a significant increase in viral DNA from 7 hpi, while exposure to a chemical inhibitor of macropinocytosis led to a significant reduction (71.6%) in viral DNA levels at 24 hpi. Our RNAseq analysis clustered the 154 HzNV-1 genes into four temporal classes. Early infection stages mostly featured replication- and transcription-associated viral genes, while late stages comprised virion assembly-associated genes. During infection, 570 host genes underwent significant up- or downregulation, mostly associated with protein folding, as well as with nuclear integrity and DNA damage responses (DDR).
Our findings support the hypothesis that HzNV-1 mainly utilizes macropinocytosis to enter ovarian cells of *H. zea*. The initiation of viral DNA replication after 7 hours matches the expression profile of replication-associated virus genes inferred from our RNAseq analysis. The modulation of the host protein folding, nuclear integrity and DDR machinery may facilitate HzNV-1 replication and assembly.

NEMATODES DIVISION SYMPOSIUM **12-1**

Past, Present and Future of *Xenorhabdus* and *Photorhabdus* bacteria

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Photorhabdus spp. and *Xenorhabdus* spp. are Gram-negative bacteria in the family Morganellaceae. They are enteric symbionts found in the alimentary canal of the infective juvenile (IJ) stage of entomopathogenic nematodes *Steinernema* spp. and *Heterorhabditis* spp., respectively. Entomopathogenic nematodes have been widely used in pest management for over four decades, and during this time, most of the studies on endosymbiont bacteria were focused on the isolation, identification and characterization of their chemical properties. However, in recent years, in addition to entomopathogenic nematodes, studies have begun to focus on the natural products produced by *Xenorhabdus*

and *Photorhabdus* bacteria. *Photorhabdus* and *Xenorhabdus* species, like all microorganisms, produce different types of soluble or volatile natural compounds during their stationary phase. Genes for synthesis of these compounds can range up to 6.5% of the bacterial genome, and these compounds are synthesized by polyketide synthetases, nonribosomal peptide synthetases, and other similar enzymes. Among compounds reported thus far are anthraquinone pigments, rhabduscin, β-lactam carbapenem, darobactin, transcinnamic acid, trans-stilbenes, phototemtide, mevalagmapeptides, and isopropylstilbenes, from *Photorhabdus* spp. Nematophin, xenorhabdin, xenortide, xenocoumacin, xenotetrapeptide. benzylidenacetone, rhabduscin, rhabdopeptide, fabclavine, ambactin, cabanillasin, indole, szentiamide, and PAX peptides from *Xenorhabdus* spp. Since 1980, much has been learned about the various biological activities of these compounds that include antibacterial, antifungal, antiprotozoal, insecticidal, molluscicidal and acaricidal effects on organisms. Such natural products are believed to be an emerging source of novel pesticides and pharmaceuticals and can serve as lead compounds for the design and synthesis of new alternatives that can replace current more toxic chemicals in the future.

NEMATODES DIVISION SYMPOSIUM **12-2**

How molecules and molecular tools have intertwined in the discovery and life history of entomopathogenic nematodes

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Undoubtedly, molecular tools and technologies have revolutionized the study of nematodes in a multidimensional manner. Their impact spans from the identification and diagnosis of cryptic species, to providing frameworks to investigate key dilemmas of their biology, ecology, physiology, and evolution. With respect to entomopathogenic nematodes (EPN), over the last 30 years, a range of molecular markers spanning from allozymes to DNA markers have been employed. Furthermore, recent developments in metaomics including transcriptomics, metabolomics, genomics are revealing not only the complexity of the symbiotic relationship between entomopathogenic nematodes and their bacterial symbionts, but also in relation to their pathogenic role in the insect host. Furthermore, CRISPR/Cas9 technologies for gene editing are currently being considered to study genetic mutations in EPN, expanding the repertoire of tools for studying parasite-host interactions. In this presentation, I will summarize the role molecular tools and techniques in the life history of EPN.

NEMATODES DIVISION SYMPOSIUM **12-3**

Bringing EPNs to higher levels – the journey for off-ground application

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Since the mid-1980s, research and development of entomopathogenic nematodes (EPNs) belonging to the families Steinernematidae and Heterorhabditidae research was initiated in Israel, along with development in other countries in North America and Europe. Several surveys were conducted to explore the abundance of EPNs populations in diverse environments in Israel. Beside the taxonomic characterization of the various populations, using molecular and morphological tools, the new isolates were subjected to a series of biological evaluations to explore their usefulness. It included infectivity and pathogenicity to various target pests and assessment of the durability of the infective juveniles (IJs) to stress conditions, mainly desiccation and heat tolerance. Also, the infectivity and efficacy of EPNs against various pests in Israel have been evaluated. The typical approach was first to assess the susceptibility of various insect stages to different EPN species in the laboratory. Further exploration of greenhouse efficacy was determined whereby tests were done with potted plants, simulating natural conditions. Field trials were conducted in commercial fields of the growers or on experimental stations. The focus was on several groups of beetles; scarabs, weevils, and flatheaded wood borers. The life history of these cryptic pests has allowed us over the years to disregard the challenges of foliar application of EPNs. Yet, phasing out of synthetic pesticides has boosted the prestige once again of microbial control in general and EPNs as well. Therefore, in recent years, we have been focusing on studying the challenges that foliage presents to EPN biology. Several formulation technologies enabled the improvement of EPNs' shortcomings of low viability and erratic infectivity. Nowadays, we are identifying the mechanism of protection rendered by formulation to EPNs IJs. These studies enable us to understand requirements from EPNs to deliver consistent and reliable results for above-ground applications.

NEMATODES SYMPOSIUM **12-4**

Advances in Formulation and Application Technology for Entomopathogenic Nematodes

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Entomopathogenic nematodes (EPNs) have been proven to be powerful microbial control agents that are used to control a wide variety of economically important pests. However, mechanisms are needed to improve control under field conditions. EPNs are formulated to enhance storage capacity and ease-of-handling. The nematodes can be applied with various application equipment. Several approaches to improve biocontrol efficacy based on application and formulation technology will be described in this presentation. Novel formulations that have recently been developed include gel-based mixtures. These innovations in formulation provide added protection to EPNs from environmental extremes such as UV radiation and desiccation. Mechanisms to improve application technology include the use of EPN-infected-hosts as the application vehicle, and the use of boosters such as ascaroside pheromones. EPN pheromones enhance nematode dispersal and infectivity. Thus, application of EPNs following pheromone exposure has resulted in superior biocontrol efficacy under greenhouse and field conditions. Further refinement and advances in formulation and application technology can be expected in the future.

NEMATODES SYMPOSIUM **12-5**

Chemical ecology of entomopathogenic nematodes: back to the future

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Within the structural and ecological complexity of the soil matrix, entomopathogenic nematodes (EPNs) must identify hosts and orientate towards them in order to achieve their life cycle. In order to do so, EPN infective juveniles (IJs)rely of various physical and chemical cues. Physical cues encompass magnetic fields or vibrations whereas volatile organic compounds (VOCs) have been proposed as effective cues

signaling the presence of an insect host and its quality. Indeed, VOCs isolated from insects triggered positive (attraction) or negative (repulsion) chemotaxis to cruiser IJs. Similarly, jumping was induced by insect isolated VOCs in nictating ambusher IJs. In addition to cues directly emitted by their hosts, EPNs respond to VOCs emitted by insect damage plant roots. These cues can diffuse over large distances (on the scale of EPNs) and are hypothesized to be alarm signals emitted by plants to attract natural enemies of insect herbivores and indirectly defend their root systems. Understanding and characterizing these interactions allowed to manipulate agroecological systems to enhanced pest management. Past discoveries and further perspectives will be discussed.

FUNGI DIVISION SYMPOSIUM **13-1**

Integrative investigations into the strategies of zombie-making *Ophiocordyceps* to manipulate carpenter ant behavior

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The evolutionary arms race between parasites and their hosts can culminate into complex extended phenotypes that benefit disease progression and transmission. The fungus-adaptive changes in behavior as seen in *Ophiocordyceps*-infected carpenter ants are a prime example. These “zombie ants” demonstrate a suite of behaviors that are thought to circumvent the social immune responses of the colony. Subsequently, the hijacked ant climbs and attaches itself at an elevated position that benefits fungal spore development and dispersal. These fungus-induced behaviors are not unique to this particular infection as parallel behaviors have also been observed in invertebrate infections by other parasite taxa. The precise mechanisms that are involved in this behavioral manipulation and others are unknown. To begin to unravel these mechanisms, we have conducted extensive fieldwork and developed the *Ophiocordyceps*-ant interaction into an integrative model system that allows us to study parasitic behavioral manipulation in greater detail in the lab. By combining fungal culturing and lab infections with behavioral assays and multiple omics approaches, we propose several comprehensive mechanistic hypotheses about the fungal proteins and ant receptors involved in this phenomenon. These hypotheses include specific fungal “manipulation” effectors of interest and their potential binding to ant proteins involved in light perception, biogenic amine binding and daily rhythms. To test these hypotheses we are currently, for the first time in this model, beginning to integrate functional genetics assays to determine the function of presumed fungal “manipulation” effectors, the host behaviors they elicit, and the host pathways that underly those phenotypes. Our results will provide detailed insights into fungus-animal interactions in general while giving some of the first insights into parasitic hijacking of animal behavior in particular.

FUNGI DIVISION SYMPOSIUM **13-2**

A biosynthetic survey of entomopathogenic fungi

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Many species of fungi establish symbioses with plants, acquiring food and shelter while protecting them from pests by producing molecules that kill or deter insect herbivores. A few of these species are used as biocontrol agents, and little is known about their pest control mechanisms. Notably, many molecules from biocontrol fungi are only produced while interacting with insects or plants, making their systematic identification using culture-based methods impractical. We use heterologous expression to systematically produce molecules from biocontrol species and are developing the capability to test their biocontrol activities.
Our laboratory has developed and validated an omics-based workflow for the discovery and cataloging of fungal molecules and biosynthetic pathways. We applied this workflow to over 100 biocontrol fungi, leading to the identification of hundreds of molecules and pathways, many of which are novel. We have established synthetic biology tools for production of polyketides and non-ribosomal peptides in fungal hosts and produced many of these molecules. Using our tools, we have developed an engineering framework for cyclic depsipeptides such as beauvericins and created new artificial variants that we are testing for bioactivity. We estimate that our workflow can lead to the production of a large fraction of the chemical repertoire of biocontrol fungi, which may help us address the challenges associated with insect pest resistance, food security, and sustainability.

FUNGI DIVISION SYMPOSIUM **13-3**

Entomopathogenic fungi and insect predators teaming up against aphids: Friend or foe?

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Exploiting the ecological networks formed by the interactions among aphids, their associated fungal pathogens, and predator fauna will give new insight for future efficient aphid control practices. Although aphid infestation in crops can be effectively controlled by either predatory ground beetles, hoverflies or entomopathogenic fungi (EPF), the interplay between these three beneficial groups is, however, unknown. Here, we present a hypothetical interaction network between aphids and their natural enemies based on three comprehensive databases compiling EPF-aphid, EPF-ground beetle, and EPF-hoverfly interactions of the European species. We aim to (1) investigate the specificity of EPF towards each group of insects, (2) assess the effect of EPF on ecosystem services provided by both carabids and hoverflies through numerical parametrization of the interaction links, and (3) investigate the role of each of the predacious agents in the dynamics of mycosis within aphid colonies. From the three separate networks linking each insect host with EPF, aphids had the highest number of EPF associations (236, 192 aphid species), then carabids (20, 12 species), and hoverflies (16, 10 species). The cross-infection network linking these three different insect hosts (214) through common EPF infection showed over 11,000 connections. Depending on EPF features, each host could potentially be a vector of EPF and can infect insects within the same guild but different taxa. How EPFs are associated with beneficial insect is less studied than that with pests, and thus we have disproportionately larger networks for aphids than for the other two taxa. Although this may hamper solid predictions of the infection pathways in real-life agricultural systems, our study helps to gain insight into the trade-off of using EPF and insect biological control agents at the same time and, ultimately, it also facilitates the adoption of suitable agricultural practices with improved biological control.

FUNGI DIVISION SYMPOSIUM **13-4**

Isolation of a probiotic bacterium to protect silkworms against fungal parasites

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Bacterial exchanges between plant phyllospheres and insect cuticles remain unclear, as does their related biological function. We found that the cuticular bacterial loads of silkworm larvae quickly increased after molting and feeding on the white mulberry (*Morus alba*) leaves. The isolation and examination of silkworm cuticular bacteria identified one bacterium *Mammaliicoccus sciuri* that could completely inhibit the spore germination of fungal entomopathogens *Metarhizium robertsii* and *Beauveria bassiana*. Interestingly, *Ma. sciuri* was evident originally from mulberry leaves, which could produce a secreted chitinolytic lysozyme (termed Msp1) to damage fungal cell walls. In consistency, the deletion of *Msp1* substantially impaired bacterial antifungal activity. Pretreating silkworm larvae with *Ma. sciuri* cells followed by fungal topical infections revealed that this bacterium could help defend silkworms against fungal infections. Unsurprisingly, the protective efficacy of D*Msp1* was considerably reduced when compared with that of wild-type bacterium. Administration of bacterium-treated diets had no negative effect on silkworm development; instead, bacterial supplementation could protect the artificial diet from *Aspergillus* contamination. Our data revealed that the cross-kingdom transfer of bacteria from plant phyllospheres to insect herbivore cuticles can help protect insects against fungal parasite attacks.

FUNGI DIVISION SYMPOSIUM **13-5**

Interaction of endophytic *Beauveria bassiana* with predators and parasitoids for hemipteran pest control in horticulture

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Biocontrol with entomopathogenic ascomycetes (EA) is a key tool to develop IPM programs to reduce reliance on synthetic pesticides, addressing environmental concerns and minimizing the development of insecticide resistance, which it is crucial in the case of the Hemipteran pests such as aphids and whiteflies, being among the most devasting pests responsible of important crop losses in protected horticulture. EA stand out among entomopathogens not only for their contact mechanism of infection through the arthropod integument, but also for developing close associations with plants including the endophytic lifestyle and rhizosphere competence that can enable them to make broader contributions to IPM and crop production. Among EA species, *Beuveria bassiana* (Balsamo) Vuill. has been shown to endophytically colonize several horticultural crops and temporally or systemically protected them against hemipteran pests and, indirectly, against other biotic and abiotic stresses. Anyhow, the interaction of *B. bassiana* with the plants incorporates multitrophic complexity at different levels including insect pests, plants, and their natural enemies. Hereby, we aimed to gather and summarize our most recent findings on the impact of endophytic *B. bassiana* on development of new hemipteran microbial direct and indirect control strategies and their integration with natural enemies, including the chemical ecology of these multitrophic interaction. On the overall, EA treatments are compatible and complementary with predators, either zoophagous and zoophytophagous, and parasitoids in terms of safety and effectiveness, with quite outstanding findings on how the chemical ecology of these multitrophic interactions favors and promote the biocontrol efficacy under semi field and farm level.

Keywords: *Aphis gossypii*, *Bemisia tabaci*, *Chrysoperla carnea*, *Aphidius colemani*, *Nesidiocoris tenuis*, melon, tomato.
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BACTERIA DIVISION SYMPOSIUM **14-1**

***Bacillus thuringiensis* at the cross roads: Insights into enteropathogenicity of *Bacillus cereus* group**

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Bacillus cereus is the name giving species of a group of genetically closely related Gram-positive endospore-forming bacteria, which is also often refered to as *B. cereus* sensu lato (s.l.)[1]. During recent years, toxigenic *Bacillus cereus* s.l. has gained prominence as an important food borne pathogen as well as the causative agent of systemic and local infections. While the relevance of the name giving species of the group, *B. cereus* sensu stricto (s.s.), as a major cause of foodborne infections and intoxications is undisputed, the role of the closely related *Bacillus thuringiensis* in food microbiology is currently under debate. *B. thuringiensis* is a widely used biopesticide, which is gaining increasing importance in frame of the ecological transformation process of food production and vector control. However, there is also growing evidence suggesting that certain *B. thuringiensis* strains can represent a food safety risk, underpinning the importance of assessing the hazardous potential of each strain used as biopesticide. Thus, this lecture will focus on *B. thuringiensis* in the spotlight of food microbiology and will also discuss the potential of novel diagnostic tools to move from the currently taxonomic driven to a more risk orientated diagnostics. In addition, the new concept of AI-powered risk negotiation[2], which allows biopesticides such as *B. thuringiensis* to be integrated into an overarching risk analysis framework for holistic health risk assessment, will be presented and discussed.

[1] Ehling-Schulz et al., 2019 The *Bacillus cereus* group: Bacillus species with pathogenic potential. Microbiol. Spectrum 6(1):GPP3-0032-2018. doi:10.1128/ microbiolspec.GPP3-0032-2018
[2] Ehling-Schulz et al., 2024. Risk negotiation: a framework for One Health risk analysis. Bull. World Health Organ. 102(6):453-456. doi: 10.2471/BLT.23.290672.

BACTERIA SYMPOSIUM **14-2**

Phenotypic heterogeneity and sporulation-independent persistence in *Bacillus thuringiensis* during infection

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Due to its exceptional resistance, the spore was long considered the unique mode of prolonged survival in sporulating Firmicutes, eclipsing any other form of persistence. The spore-forming entomopathogenic bacterium *Bacillus thuringiensis* (Bt) was shown to complete its infectious cycle by sequentially activating virulence, necrotrophism and sporulation (Spo) genes. Although these processes are controlled by quorum-sensing systems, the bacterial population was heterogeneous in the host cadaver, and spores constituted only 30% of the bacterial load. We investigated the behavior of the bacterial population and the characteristics of the Spo$^-$ form in the Bt/*Galleria mellonella* infection model. Using fluorescent reporters and molecular markers coupled to flow cytometry and microscopy, it was demonstrated that the Spo$^-$ cells constitute about half of the population two weeks post-infection (pi).

Protein synthesis and growth recovery assays indicated that they are in a metabolically slowed-down state. They were also found to be resistant to the cadaver environment, which did not support growth of *in vitro*-grown vegetative cells and spores. A transcriptomic analysis of this subpopulation at 7 days pi revealed a signature profile of this state, and the expression analysis of individual genes at the cell level showed that more bacteria mount an oxidative stress response as their survival time increases, in agreement with the increase of the free radical level in the host cadaver and in the number of reactive oxygen species-producing bacteria. Altogether, these data indicate that non-sporulated bacteria engage in a profound adaptation process that leads to their persistence in the host cadaver. Phenotypic heterogeneity of the Bt population during infection and, in particular, coexistence of spores and non-sporulated forms able to survive for a prolonged period in a host cadaver, could confer this bacterium advantages that still need to be elucidated.

BACTERIA SYMPOSIUM **14-3**

Assessing insecticidal protein safety without HOSU or source organism

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The use of History of Safe Use (HOSU) information, such as origin from the soil bacterium *Bacillus thuringiensis* (Bt) that has been widely utilized in agriculture for insect pest control, has been an integral component of the Weight-of-Evidence approach in the protein safety assessment of genetically modified (GM) insect-protection crops. However, as new types of insecticidal proteins, often derived from non-Bt sources are being explored for the development of next-generation products, the availability of HOSU information can become lacking due to the novelty of these sources. This presentation will introduce the current framework for protein safety assessment in GM crops, provide a historical perspective on HOSU considerations, and discuss potential paradigm shifts in assessing the safety of next generation insect protection crops.

BACTERIA SYMPOSIUM **14-4**

Towards a consensus taxonomy of the *Bacillus cereus* group of bacteria in the sequencing era

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A robust microbial taxonomy (naming/description/classification) has profound implications for understanding pathogenicity, and effectively setting appropriate legal and regulatory frameworks. Thus, objectively delimiting microbial species boundaries remains an important challenge and becomes urgent when unresolved taxonomy threatens food security and public health. An example of this is the taxonomic situation for the *Bacillus cereus* group. These closely related Gram-positive spore-forming bacteria include the opportunistic pathogen *B. cereus* (*sensu stricto*), frequently implicated in food poisoning and food spoilage worldwide, *Bacillus anthracis*, the cause of the lethal disease anthrax, and *Bacillus thuringiensis*, an insect pathogen which is the world's most widely used biological pesticide (sprayed on crops to kill insect pests). These three species are largely defined by their toxicity phenotypes. Over the last decade, however, 30 additional species, and even up to 57 genomospecies, have been proposed in the group based on numerous genetic analyses, leaving the *B. cereus* group taxonomy in collapse. *B. cereus* group bacteria are thus at the centre of a taxonomic conflict, as the different "species" are hard to identify based on genetic criteria and, at the same time, the main distinguishing phenotypic (toxicity) features are conferred by mobile genetic elements (MGEs). A novel approach for a nomenclature of the *B. cereus* group was recently proposed, based on a phylogenetic genomospecies/subspecies/biovars framework. However, this novel classification remains controversial and has not been generally or formally adopted. We have started a collaborative effort to constitute a research network that addresses the issues of *B. cereus* group taxonomy, accommodating the phylogenetic approach and reconciling the contribution of MGEs, while still retaining some of the clinical, environmental, and socioeconomic aspects associated with the classical species.

MICROBIAL CONTROL DIVISION SYMPOSIUM **15-1**

Getting more for your money. Can we exploit interactions of microbial and chemical pesticides for increased pest control?

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There is an increasing drive to use multiple, integrated pest control strategies, limiting chemical application and increasing the use of sustainable alternatives for plant protection. Greenhouse whitefly (*Trialeurodes vaporariorum*), a major global pest, cause direct damage to >850 plant species and transmit viral plant diseases. Management of *T. vaporariorum* is increasingly difficult because of widespread pesticide resistance. Many greenhouse growers rely on biological control agents to maintain *T. vaporariorum*. However, biological control agents are slow acting and variable in their efficacy. Co-application of a chemical insecticide with entomopathogenic fungi (EPF) can result in improved pest control, assist resistance management, target multiple pest species and increase the range of environmental conditions over which control is effective. Combining chemical and biopesticides has the potential to result in both positive and negative interactions, so it is important to understand how mixture components interact. Positive interactions result in synergism, whilst negative interactions cause antagonism and a reduction in pest control. EPF with potential to control *T. vaporariorum* were identified through a series of laboratory in vitro and in vivo tests. Co-application of a mixture of selected EPF and the chemical insecticide spiromesifen were evaluated in laboratory-based bioassays. Using an ecotoxicological MixTox model, interactions between the EPF and insecticide were described; depending on the EPF and the concentrations applied, mixtures resulted in additivity, synergism or antagonism. The types of interactions were influenced by temperature and applications of a low concentration of spiromesifen with EPF resulted in additive mortality of *T. vaporariorum* in greenhouse trials. Only by understanding the complex interactions between components of integrated pest management strategies can optimised approaches be developed to control pests.

Testing Microbial Pest Control Products as components of IPM programs: considerations for appropriate trial design

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In practice, plant protection products are often recommended to be applied as a component of a plant protection programme, especially for the control of difficult and 'long-season' target pests. In these cases, efficacy trials allowing for the evaluation of the effectiveness of a plant protection product when it is a component of a programme can be useful to assess the practical value of the same plant protection product. Such trials are also useful for the evaluation of the effectiveness of plant protection products which are approved with a very limited number of applications per season while, for effective control of the target pest, several applications could be required. Furthermore, a conventional design based around randomized small plots, replicated treatments and direct comparisons with reference products and untreated controls is not practical for some pests, especially soil pests and soil-borne pathogens (i.e. highly-aggregated non-homogeneous pests), or for pests capable of moving over long distances and over a long period of time. Due to their biology, a large-plot trial layout would be more suitable.
Examples of trial design and layout to be used in randomized small-plot trials and non-randomized large-plot trials for evaluating the effectiveness of plant protection products in plant protection programmes are provided.

Future position of Biocontrol in Integrated Pest Management

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The good practice model for plant protection is founded on Integrated Pest Management (IPM). Biocontrol (microbials, botanicals, semiochemicals, natural enemies) is increasingly taking a leading role as the main suite of interventions in IPM and is an economically feasible way to manage pest populations. Within IPM, Biocontrol interventions are used in a binary way, as are conventional chemical pesticides: there is a direct relationship between pest problem and solution. Considering how plant protection and IPM is developing, there is increasing evidence that tertiary interactions are being successfully evaluated and implemented into IPM. What is less clear is what plant protection will look like, if each layer of good practice IPM fully utilises the biological and supporting (digital, sensing, formulation, application) tools available to farmers.
This presentation considers how Biocontrol interventions will continue to be used and how good practice, bio-intensive IPM is already evolving and capitalising on the biology and ecology of the products available. It will also explore evidence for a new plant protection model and the role of Biocontrol in this.

Challenges of pathogens detection in insect mass rearing: Advances from the Insect Doctors program for improved diagnostic

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For any given pathogen, the **onset of epidemics** primarily depends on the opportunity for contact with susceptible hosts, and for a focal infected host, the **onset of disease** depends on the dose and virulence of the infectious agents. However, for any host pathogen interaction these seemingly simple parameters may vary in function of biotic and abiotic environmental conditions. Farms are ideal breeding grounds for pathogens because they keep hosts in enclosed environments and increase their density. Insect mass rearing for biocontrol application or feed and food is fast expending worldwide. However, pathogen emergence does happen in these facilities holding large colonies in closed environments, and disease outbreaks can have major economic impacts, as experienced for centuries in silkworm production and more recently by cricket breeders. To prevent diseases, and **promote health and welfare**, it is thus important to diagnose pathogens as early as possible. However, depending on the focal host species, several challenges may arise. Here I will review some of the advances in pathogen discovery made during the Insect Doctors program.

Emerging Threats to Black Soldier Fly Farming in Africa

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Black soldier fly (BSF) [*Hermetia illucens*] farming is presently the most widespread form of insect farming in the world. Despite technological developments and regulatory improvements, increase production has lead to many novel challenges and uncertainties, including problems with diseases that have potential to cause severe colony collapse and decline. In Africa, research efforts to identify such diseases have received limited attention, though they are a bottleneck for every type and scale of BSF production system for feed. We also include a few examples of parasitoids and mites, which are not (yet) reported in other parts of the world to increase our understanding of selected factors that might be potential triggers for BSF colony decline and for the development of disease prevention and control measures. Diseased BSF colonies in Ethiopia and Uganda revealed causal pathogen(s) such as bacteria, fungal and viral pathogens using high throughput metabarcoding and transcriptomics. Further a newly described pupal parasitic wasp [*Eniacomorpha hermetiae* Delvare] was observed to cause a significant reduction (>70%) in the emergence rate of BSF. Phoretic mites [*Macrocheles muscaedomesticae*] are posing an emerging threat to BSF farming, directly affecting flights, movement and causing exhaustion or death to the flies. Parasitism by the mites significantly reduced mating success of males, egg laiding by the females and life span [3.2 – folds and 2.4-folds for female and males, respectively]. Based on these findings, recommendations towards possible future research directions with possibilities to enhance the resilience of BSF production to disease or other outbreaks are crucial. This study provides critical information necessary to mitigate colony decline and enhance BSF production in Africa and globally.

Diseases in shrimp aquaculture

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It is well known that global aquaculture production has increased and in recent decades has surpassed capture fisheries as a source of aquatic animal protein. A major limiting factor in the sustainable growth and development of the aquaculture industry is disease. In shrimp aquaculture, for example, infectious disease outbreaks are considered one of the principal limiting factors to increased production. High profile disease events in the $15bn shrimp industry include those caused by White Spot Syndrome Virus (WSSV), the bacterial pathogen implicated in Acute Hepatopancreatic Necrosis Disease (AHPND) and emergent pathogens such as *Enterocytozoon hepatopenaei* (EHP); these pathogens are implicated in annual losses of $3bn per annum. The trade of live aquatic animals and their products is known to be a major factor in disease spread and emergence, for example via movement of infected stocks between farming regions. New diseases will continue to emerge with the detection of previously unknown pathogens and are expected to increase in parallel to the expansion of the aquaculture industry. There is often a lag phase between the development and the subsequent detection of a new disease/pathogen. To mitigate the effects of these infectious diseases it is critical to rapidly detect and characterise the causative agent(s), develop accurate diagnostic tools, understand their epidemiology, and to disseminate the information efficiently to raise awareness to facilitate control measures. I will present an overview of known diseases within shrimp aquaculture, detailing their emergence, discovery and impacts upon shrimp production globally.

Microsporidia and protist parasites in reared insect hosts

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The global rearing of invertebrates for food and feed faces significant challenges from diseases caused by diverse pathogens, including microsporidia and protists. A literature review of the diversity of these parasites in reared insect species highlighted major entomopathogenic groups such as Amoebozoa, Apicomplexa, Ciliates, and Microsporidia in these hosts. Utilising Nanopore sequencing with group-specific primers and innovative amplicon pooling strategies, novel genomic data were generated to enhance phylogenetic relationships and improve diagnostic tools. This study underscores the importance of advanced molecular techniques in managing pathogen impacts and ensuring the health and sustainability of reared insect populations. Eventually, to assess the potential importance of gregarines and microsporidia as ubiquitous parasites in crickets, the effects on different life traits of two cricket hosts, *Acheta domesticus* and *Gryllus bimaculatus*, were investigated in laboratory bioassays.
Keywords: Nanopore, micro-eukaryotes, parasites, reared insects, bioassays

Worldwide microbial pest control agents or environmentally competent ones?

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Whilst there is a common agreement in that mortality, production efficiency, and safety are three decisive selection criteria for a commercial microbial insecticide, in many regulatory frameworks, the active ingredients (the microbial strains), are approved and authorized (formulated products) according to the systems initially developed for chemical pesticides that has led to not only to a slow implementation of microbial control due a time-consuming and costly schedule, but also a global trend for the use of registered strains worldwide, and not in uniform climatic zones, with low attention given to their sensitivity to abiotic and biotic factors governing in each region, and hence, to the use of microbial strains that lack environmental competence. This is particularly important in a climate change scenario in which the relationships between insect pests and their entomopathogens can be highly altered. The lack of environmental competence of the microbial active ingredients has very seldom encouraged farmers to believe that microbials are unable to protect their crops. Appropriate microbials mass production and formulation strategies can partially solve the fitness and viability of the infective propagules, whereas the only strategy to guarantee high virulence and infectivity is selection of environmentally competent microbial strains that are able to persist in the host environment for the required infection period. Hereby, the criteria for selection of environmentally competent microbial strains are examined for the different groups of entomopathogens. In general, UV radiation is a key factor for propagule depletion and inactivation in epigeal habitats, while temperature, followed by humidity are the is most critical factors for reducing the infectivity and virulence of entomopathogens in epigeal and hypogeal habitats, whereas special and increasing attention should be given to the microbiota in the later habitats. New rules for registration of microbials control agents could be a boost for the development and commercialization of microbial control solutions adapted to relatively uniform climatic zones.
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From Legacy to Weapon: Unveiling a New Biopesticide

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Professor Yoel Margalith, the visionary behind Bti, left behind a remarkable legacy: a collection of 500 potentially potent pest control bacterial isolates. This narrative recounts the captivating journey of how academic research meticulously screened and evaluated these strains, ultimately identifying one with exceptional promise. Guided by this discovery, Biodalia Microbiological Technologies took the baton, navigating the steps of scaling up to industrial production, registration processes and documentation, and finally, commercialization. However, this path wasn't paved with ease. Countless challenges tested our resolve, pushing us to innovate and collaborate.
Now, we stand ready to share our captivating story, a tale of unlocking the potential from Professor Margalith's legacy and presenting a groundbreaking new weapon against pests
This lecture explores Biodalia's journey transforming Prof. Margalith's bacteria into a groundbreaking new weapon against agricultural pests.

Innovative mass production and novel formulation of baculovirus biopesticides for false codling moth management

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The Cryptophlebia leucotreta granulovirus (CrleGV) has been used commercially for the control of the false codling moth (FCM), *Thaumatotibia leucotreta*, for 20 years. FCM is an important pest of citrus and other crops in sub-Saharan Africa and Israel, thus with quarantine status for export markets. Although the CrleGV products have been shown to work well, there were four challenges that needed to be addressed to improve management of the pest. These were a) the possibility of resistance development, as experienced with codling moth against the Cydia pomonella granulovirus (CpGV) in Europe and more recently the USA, b) improvement of virulence against all populations of the pest, c) broader host range efficacy, where FCM and one or more of its close relatives attack a specific crop, and d) improved formulation for greater field efficacy. We report how this has been achieved through the development of a CrleGV biopesticide consisting of more than one isolate, the discovery and formulation of a novel broad-host range nucleopolyhedrovirus and an encapsulation formulation of occlusion bodies in a preparation consisting of nano and micro vesicles and sponges that act as bio-transporters.

Back to Basics: Reconciling Comflicting Taxonomic Practices

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Taxonomy and nomenclature are wholly different concerns, and major changes introduced to fungal nomenclature in 2010 led to rapid divergences of practice and opinion about the naming and classification of fungi using traditional approaches based on morphology, development, and a host of other readily discernible features as opposed to phylogenetic and genomic based approaches. The 2010 changes rejected the long-standing dual nomenclature for pleomorphic fungi (mostly ascomycetes) that allowed valid but separate names for their sexual and asexual states–that rarely appear togerther in time or space–to adopt a rule that any fungus (plus many algae and, inexplicably, fossil plants!) can bear only one valid name that must be applied to all of its life stages. Hypocrealean ascomycete entomopathogens were DEEPLY impacted by this new (1F=1N, One Fungus=One Name) rule, but other fungal entomopathogens also have seen new taxonomic controversies under the current rules. These new rules effectively force the use of sequence data to recognize relationships and to organize taxonomies (while also too often ignoring much key data gained by traditional taxonomic methodologies). Secondary effects of the 1F=1N rule (1) negatively affect basic mycological teaching; (2) drive a trend to inflate perceived taxonomic differences to ever higher ranks in the hierarchy (further complicated by the fact that ranks above the family are not subject to priority rules); (3) too often ignore the importance of and required dependence on the rules of typification (and knowledge of the many types of types); (4) failure to recognize how small a proportion of the sequence information in the databases is based on type or verifiably identified source organisms; and (5) identifying unknown fungi by matching sequences with potentially misidentified source organisms risks a real dangers of Garbage In/Garbage Out result for those proposing new taxa and new taxonomies.

Understanding the environmental context of entomopathogenic fungi: Fungal community dynamics surrounding mycosed invertebrates and isolation of new entomopathogenic fungal species

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Infected spiders showing fungal sporulation on their bodies were characterized from a forest region in central Florida. Molecular sequencing and phylogenetic reconstructions using four genomic, namely internal transcribed sequences (ITS), large subunit ribosomal RNA (LSU), small subunit ribosomal RNA (SSU), and translation elongation factor-1 (TEF1) regions, revealed one isolate, UFSI_5, forming a distinct separate branch within *Gibellula*, and two isolates, UFSI_3 and UFSI_4 essentially matching *Parengyodontium album*. Morphological characteristics of the isolates, including growth and elaboration of fungal structures were examined. Microscopic observation of the parasitized cadavers showed on the macro level that the fungal structures on the spiders from which *Parengyodontium* was isolated and those on the spider from which *Gibellula* was isolated were similar in appearance. However, during growth on standard mycological media, the *Gibellula* isolate appeared to form rudimentary fruiting structures and purple-pigmented spores, whereas the *Parengyodontium* isolates were primarily white and smooth, but with yellowish pigmentation of the hyphae and a slight darkening of the media surrounding colonies. To determine the extent to which these fungi could be found in the surrounding environment, sampling and subsequent ITS Illumina based amplicon sequencing of the soil, leaf litter, and above ground plant tissues within the immediate vicinity of the infected cadavers was performed. Fungal diversity at these different trophic levels were characterized. These data combine invertebrate pathogen identification via characterization of infected specimen with surrounding environmental sampling to provide a more complete picture of the ecological context of these fungi.

A brief history of *Beauveria bassiana and Metarhizium anisopliae*: Resolving historical problems and anticipating future challenges

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Beauveria bassiana (Bals.-Criv.) Vuill. and *Metarhizium anisopliae* (Metschn.) Sorokīn are globally ubiquitous entomopathogenic fungi and key model systems for fundamental and applied research in insect pathology and insect biological control. We briefly trace the taxonomic histories of both species, illustrating how their species concepts have evolved within the changing landscape of taxonomic practice and the emergent role of molecular biology in driving insights into their evolutionary history and population biology. Although understanding of both these insect pathogenic species has been clarified over time, the current taxonomic status and concepts of the two species present two contrasting challenges. *B. bassiana*, while properly typified, encompasses an extensive cryptic species complex and questions arise as to whether further taxonomic division is warranted. In contrast, while molecular phylogenies of *M. anisopliae* conform closely to the definition of a unitary phylogenetic species, it is not properly typified. Thus, its species concept remains vulnerable, and we propose to epitypify the

species to preserve its current phylogenetic species concept. We discuss the importance of valid typification to ensure stable taxonomic species concepts and discuss future research directions to investigate the role(s) of sex, extended clonality, and geography in better understanding the genetic composition and structure of these two important insect pathogenic species.

WORKSHOP NEMATODES AND FUNGI DIVISIONS **18-4**

Addressing Taxonomic Vandalism in Entomopathogenic Nematodes: Reflections on Past Successes and Future Challenges

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Taxonomic vandalism presents a pervasive challenge across various scientific disciplines, undermining the integrity of biological classification systems and hindering our understanding of biodiversity. In entomopathogenic nematodes (EPNs), taxonomic classification traditionally relied on morphological characteristics, later augmented by crossbreeding tests and standardized description methods outlined in protocols by Hominick et al. in 1997. The systematics of EPNs has undergone a significant transformation with the advent of molecular methods. While these new tools have largely validated previously identified EPN species and facilitated a substantial increase in the number of recognized species they have also introduced challenges. These include describing novel species using insufficiently curated or short sequences, or using erroneous sequence alignments. Subsequent studies have addressed taxa lacking adequate molecular support by synonymizing them with previously described species. Presently, the EPN community has largely reached a consensus on species concepts and a species list comprising over 130 recognized taxa, fostering a transparent systematics framework compared to other nematode groups. Nonetheless, challenges persist, particularly concerning species descriptions lacking sufficient molecular validation and authors failing to deposit type material in public collections. These issues could be avoided by a rigorous revision process and a vigilant and cautious community of reviewers in the EPN field.

WORKSHOP NEMATODES AND FUNGI DIVISIONS **18-5**

Current methods for the taxonomy and systematics of the entomopathogenic bacterial genera *Photorhabdus and Xenorhabdus*

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Entomopathogenic bacteria of the genera *Photorhabdus* and *Xenorhabdus* establish obligate symbiotic relationships with nematodes of the genera *Heterorhabditis* and *Steinernema*, respectively. The nematodes infest soil-dwelling small arthropods, including insects. Right upon infestation, the nematodes release their symbiotic bacterial partners, that produce a plethora of pathogenic factors that kill the infected organism. The diversity of both nematodes and bacteria is enormous. The genus *Photorhabdus* contains 30 taxa (23 species, 6 of which are divided into different subspecies), the genus *Xenorhabdus* contains 32 taxa (31 species, 1 of which is divided into two subspecies), the genus *Heterorhabdtiis* contains 22 species, and the genus *Steinernema* more than 100 species. During my talk, I will focus on different aspects of the taxonomy and systematic of the bacteria. In this context, I will give an overview on the current taxonomic status of the genera *Photorhabdus* and *Xenorhabdus*, including the experimental tools that are currently used to classify the different species of these two genera.

POSTER SESSION **B-P1**

Receptor recognition site of insecticidal pore-forming toxin Cry46Ab

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Cry46Ab is a protein produced by the soil bacterium Bacillus thuringiensis (Bt) and is toxic to mosquito larvae and apple snails. Mosquitoes transmit infectious diseases such as dengue fever and Zika fever. The apple snail is a pest of rice in East Asia and Southeast Asia. Cry46Ab is thought to be useful in the development of biopesticides to exterminate these harmful organisms.
Three-dimensional structural analysis of Cry46Ab reveals that this protein is an aerolysin-type β-type pore-forming toxin (β-PFT) and consists of three domains. A structural comparison with other β-PFTs suggests that domain I contains a target cell recognition region involved in receptor binding. Therefore, we created amino acid substitution mutants of the two aromatic amino acid clusters in domain I of Cry46Ab and compared their insecticidal activity against mosquito larvae. Among these mutants, the single amino acid substitution mutant retained flea killing activity. However, a combination of two amino acid substitution mutants that lost insecticidal activity was found.

POSTER SESSION **B-P2-STU**

The Construction of Secretory Expression Engineering Bacteria for the Trans-Cry3Aa-T-HasA Fusion Protein against the *Monochamus alternatus*

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Pine wood nematode disease is currently the most deadly forest disease in China, and the *Monochamus alternatus* is its primary vector. Controlling the *M. alternatus* is crucial for managing pine wood nematode disease. This study, based on the selected HasA (pGHKW4) secretory expression vector, used electroporation to combine the genetically modified high-toxicity toxin Cry3Aa-T with the entomopathogenic bacterium *Yersinia entomophaga* isolated from the gut of the *M. alternatus*. The SDS-PAGE and Western blotting techniques were employed to confirm the toxin protein's secretion capability. The engineered bacteria's genetic stability and effectiveness in controlling *M. alternatus* were assessed for their insecticidal activity. The results of the SDS-PAGE and Western blotting analyses indicate that the HasA system effectively expresses toxin protein secretion, demonstrates certain genetic stability, and exhibits high insecticidal activity against *M. alternatus*. This study constructed a highly toxic entomopathogenic engineered bacterial strain against *M. alternatus* larvae, which holds significant implications for controlling *M. alternatus*, laying the foundation for subsequent research and application of this strain.

POSTER SESSION **B-P3-STU**

Potential of feed-born bacteria for phytopathogen and insect pest management

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Biotic adversities affecting crops intended for animal feed cause significant economic damage, which requires sustainable and eco-friendly measures to contain populations of microbial plant pathogens and insect pests. In the frame of the activities of SPOKE 03: "APPàre: smart and secure livestock farm APPlications to boost data-driven innovation along the food chain - AgriVet" (Project e.INS Ecosystem of Innovation for Next Generation Sardinia, PNRR), one of the approaches pursued is to collect microbes living in these feed matrices in order to assess their potential use as natural antagonists of harmful species. For this purpose, new bacteria were isolated from animal feed (silage and wrappers) and from animal digestive tract (rumen). Such bacteria were preliminarily subjected to identification by 16S rDNA sequencing, which allowed to identify a variety of species in the genera *Bacillus*, *Brevibacillus*, in addition to several lactic acid bacteria (LAB). An initial screening was dedicated to assessing in vitro the antimicrobial properties through plate bioassays against major plant pathogens (*Fusarium*, *Aspergillus*, and *Verticillium* species). Secondly, bacterial suspensions and their culture supernatants were assayed by contact and ingestion on model insects including coleoptera (i.e., mealworms) and diptera (i.e., fruit flies). Accordingly, some of the bacterial isolate was significantly effective against these targets with a dose-dependent mechanism. A selection of the most effective isolates is being characterized by biochemical and molecular analyses, in order to identify the main virulence factors and the mode of action, specifically targeting the insecticidal proteins.

POSTER SESSION **B-P4-STU**

Bacterial entomopathogens for the management of insecticide-resistant mosquito populations

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Mosquito-borne diseases are a major health concern worldwide. While biocides have successfully been used to contain their populations, the situation appears critical globally. The development of insecticide resistance, in addition to climate change and people's movement across continents, are favouring the establishment of pest species in new habitats and the spread of diseases in new areas. Though the use of chemical insecticides remains the easiest strategy to implement, especially to face emergency conditions, the search for low environmental impact approaches is highly desired. Ecologically friendly alternatives include the application of insect growth regulators and bacterial entomopathogens, such as *Bacillus thuringiensis israelensis* and *B. sphaericus*, in immature development sites. In the frame of the regional plan for monitoring insecticide resistance in vectors implemented in Sardinia Island (Italy), populations of the two main mosquito species, *Aedes albopictus* and *Culex pipiens*, are being genotyped for their resistance to biocides. Mosquito colonies have also been established in the laboratory of the Experimental Zooprophylactic Institute of Sardinia and the mosquitocidal potential of a range of bacterial species with a known ecotoxicological profile has been assayed. This includes novel and promising strains in the genera *Bacillus*, *Brevibacillus*, and *Pseudomonas*. Local mosquito populations with different insecticide resistance profiles have been targeted.

POSTER SESSION **B-P5**

Nanoparticle-loaded microcapsules providing effective UV protection for Cry protein

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UV irradiation destroy the three-dimensional structure of the crystal protein, which directly affect its solubility in alkaline solutions and insect midgut. To develop UV resistant microcapsules of Bt crystals for improving the stability of Bt insecticides, a novel microcapsule of Cry1Ac parasporal crystals was explored. Microcapsules were prepared by the layer-by-layer (LbL) self-assembly technique and electrostatic adsorption of nanomaterials. Chitosan and sodium alginate were used as the coating materials, and nano-ZnO, nano-SiO_2, nano-TiO_2 were used as UV protective agents adsorbing on the surface of the microcapsule. The morphology, stability under UV radiation and bioassay were studied respectively. Scanning electron microscopy (SEM) showed that three kinds of nanoparticles could adhere successfully to the surface of microcapsules. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and bioassay was used to test the changes of protein and insecticidal activity after UV irradiation. Our results suggested that all of the three nanoparticles could effectively protect Cry1Ac from UV rays. Among them, nano-ZnO was the strongest, followed by nano-SiO_2, while nano-TiO_2 was not as good as that of the former two. It is a successful attempt to improve the UV resistance of Bt microcapsules by directly adsorbing nanoparticles on the outest layer. The preparation is expected to be a new anti-UV formulation of Bt after further optimization.

Keywords: *Bacill us thuringiensis*; microcapsule; nanoparticles; UV resistance；insecticidal activity

POSTER SESSION **B-P6**

NupR is involved in the control of PlcR, a pleiotropic regulator of extracellular virulence factors

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PlcR is a pleiotropic regulator of extracellular virulence factor gene expression in *Bacillus thuringiensis* (Bt). It was reported that PlcR activates its own expression while Spo0A-P represses its transcription. In this research, we demonstrated that NupR, a nucleoside permease regulator belonging to the GntR family, can directly bind to a conserved binding site in the 5' non-coding region of *plcR* and repress its expression in the stable phase. Glucose is known to induce the expression of *nupR* and indirectly inhibit the expression of *plcR* through NupR. However, after adding glucose at the logarithmic end, the transcription level of *plcR* significantly increased in both wild and *nupR*-deficient Bt strains. It increased even more in the deficient strain. These results suggested that glucose may inhibit the production or accumulation of SpoOA-P, leading to an increase in the expression of *plcR*. In addition, guanosine, uridine, cytidine, and adenosine can all induce the expression of *plcR*. In short, *plcR* is negatively regulated by NupR during the stable phase. At the beginning of the stable phase, additional nucleosides can induce the expression of *plcR*, thereby affecting the expression of virulence factors such as bacterial enterotoxins, hemolysin, and metalloproteinases. This study reveals a novel regulator for *plcR* expression. It combines cell nutritional status with virulence to form a regulatory loop, providing new ideas and research foundations for studying bacterial virulence.

POSTER SESSION **DBI-P1-STU**

Flagella of *Paenibacillus larvae* – influence on multicellular behavior and virulence

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American Foulbrood (AFB) is one of the most devastating diseases of the honey bee brood, posing a threat to the health of bee colonies worldwide. The causative agent of AFB is *Paenibacillus larvae*, a Gram-positive, spore-forming bacterium that is classified into different genotypes (ERIC I-V). Only *P. larvae* ERIC I and ERIC II are responsible for current AFB outbreaks among these genotypes. AFB infection begins when the ingested spores of *P. larvae* germinate in the midgut lumen of young honey bee larvae. Following a massive proliferation of vegetative bacteria in the midgut, *P. larvae* invades the midgut epithelium, subsequently infiltrates the haemocoel resulting in the death of the larva. The bacteria decompose the larval carcass into a ropy mass that dries into a tightly adhering scale, consisting of billions of spores. During the infection cycle various genotype-specific virulence factors, as well as multicellular behavior like swarming motility and biofilm formation, help the bacteria to colonize its host. Furthermore, *P. larvae* synthesizes peritrichously arranged flagella on its cell surface. These bacterial appendages are known not only to mediate the motility of individual planktonic cells, but also to play an important role in swarming motility and biofilm formation.
To enhance our understanding of AFB pathogenesis, we investigated whether the flagella of *P. larvae* ERIC I and ERIC II influence multicellular behavior and virulence of the bacteria. We discovered mutations in two flagellar biosynthesis genes of *P. larvae* ERIC I presumably causing the non-swarming phenotype previously described for this *P. larvae* genotype. Furthermore, we discovered that the flagella of both genotypes are essential for biofilm formation and full virulence, thus identifying the flagellum of *P. larvae* as a virulence factor. These findings provide further insights into AFB pathogenesis and could be of significance in the fight against this devastating honey bee disease.

POSTER SESSION **DBI-P2**

Epidemiological study of *Paenibacillus larvae* via MLVA using the example of a major European city

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The causative agent of American Foulbrood (AFB), a globally occurring disease of honey bee larvae, is the Gram-positive bacterium *Paenibacillus larvae*. Various genotyping methods exist for *P. larvae*. The first protocol developed for genotyping was repetitive element PCR (repPCR) performed with Enterobacterial Repetitive Intergenic Consensus (ERIC) primers, which led to the identification of several ERIC-genotypes. The next genotyping method developed for *P. larvae* was Multi Locus Sequence Typing (MLST). The established MLST scheme, which is based on the analysis of seven housekeeping genes, identified 48 *P. larvae* MLST types which grouped according to the ERIC classification scheme, thus confirming and extending it, but at the same time also suggesting considerable differences in the genetic variability between the two genotypes. In order to analyze this assumed difference and achieve a better resolution of the genetic variability of *P. larvae*, we established and optimized a PCR-based method that analyzes the

variable number of tandem repeats (VNTR) in 11 regions of the *P. larvae* genome, which is called multiple locus VNTR analysis (MLVA).
By analyzing more than 1,000 *P. larvae* strains with this optimized method, we identified more than 300 MLVA types. The classification into the ERIC genotypes remained unchanged, but it became clear that the two practically relevant genotypes, ERIC I and II, exhibit similar genetic variability, which could not previously be demonstrated by MLST analyses.
Using the example of our AFB-monitoring in Berlin, we can demonstrate the power of this method for the molecular epidemiology of *P. larvae*. Samples from the years 2002-2023 were analyzed and the MLVA types of the isolated *P. larvae* strains were determined. The location data of the analyzed samples allowed a geographical evaluation of the epidemiological situation of *P. larvae* in all 12 districts of Berlin over 20 years.

POSTER SESSION **DBI-P3-STU**

Establishment of an exposure bioassay for experimental infection of honey bee larvae with SBV

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Honey bee viruses have attracted considerable interest over the last two decades and it is becoming increasingly clear that viral infections play an important role in the deterioration of bee health. More than 20 viruses infecting honey bees have been identified, but research mostly focusses on three of them, deformed wing virus (DWV), Chronic bee paralysis virus (CBPV) and viruses belonging to the AKI complex (Acute bee paralysis virus, ABPV; Kashmir bee virus, KBV; Israeli acute paralysis virus, IAPV). Among the rather neglected viruses in Europe is sacbrood virus (SBV). This neglect could be due to the fact that, although SBV can be quite frequently detected in adult bees in apparently healthy colonies, it rarely causes symptoms and cannot be linked to colony losses. The lack of basic research on this virus also means that the pathogenesis of SBV infections is not yet understood. In order to change this dire situation and to bring this neglected virus to the fore, we have developed an exposure bioassay for the infection of larvae with SBV as a first step towards in depth analysis of this virus. Here, we will present our data on the development, establishment and validation of this SBV exposure bioassay.

POSTER SESSION **DBI-P4-STU**

Evolution of virulence in *Paenibacillus larvae*, a honey bee pathogenic bacterium

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Paenibacillus larvae is a Gram-positive, spore-forming bacterium and the causative agent of the notifiable epizootic disease American foulbrood (AFB). The larvae of the western honey bee (*Apis mellifera*) are the only known hosts for *P. larvae*, indicating that this bacterium is a highly specialized obligate pathogen. The infection cycle shows that *P. larvae* is adapted to the eusocial behaviour of honey bees, as the spread of spores is linked to the hygiene behavior of the bees. An infection starts when first instar larvae ingest food contaminated with *P. larvae* spores, which then germinate in the midgut. Massive proliferation is eventually followed by an infiltration of the haemocoel, causing the death of the larvae. *P. larvae* decomposes the larval cadaver to a ropy mass, which dries down to the so-called foulbrood scale containing millions of newly generated spores. These spores are the source of intra- and intercolonial horizontal transmission.
To understand the evolutionary processes in *P. larvae* during its recurring interaction with the larval host, we established an *in vivo* assay allowing to serially passage *P. larvae* through honey bee larvae, mimicking the natural infection cycle in a colony. Spores were isolated from foulbrood scales, counted and used for the next round of infection. Statistical analyses were used to determine whether the survival rate changed systematically during the evolution experiment. Whole genome sequencing was performed on strains isolated from three different passages to assess the occurrence of mutations. Analysis of the survival rate showed that total mortality due to *P. larvae* infection varied during the serial passage experiments. Comparative whole genome analysis of *P. larvae* isolated from the passages revealed distinct deletions which appeared to correlate with the observed variation in larval mortality. This suggests that selective pressure is working against the loss of relevant genes.

POSTER SESSION **DBI-P5-STU**

The necrotrophic lifestyle of *Paenibacillus* larvae: Suppression of carcass microbiota in honey bee larvae

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The gram-positive and spore forming bacterium Paenibacillus larvae is the causative agent of American Foulbrood, a worldwide occurring disease of the honey bee brood. Honey bee larvae become infected by ingesting food contaminated with P. larvae spores. Ingested spores germinate in the larval gut and after massive proliferation, the vegetative bacteria kill the host by invading the hemocoel. The larval carcass is decomposed by P. larvae into a ropy mass, which dries into the characteristic foulbrood scale accompanied by the sporulation of P. larvae to form the next generation of infective spores thus finalizing the spore-to-spore infection cycle. It is mandatory for the success of this infection cycle that a pure culture of P. larvae is established after the death of the infected larva. Our previous work has shown that P. larvae encounters microbial competitors not before the death of the infected larva, hence, not before ingested spores of various saprophytes germinate in the carcass of the larva. We hypothesized that Sevadicin and Paenilamicin, two antimicrobial secondary metabolites synthesized by P. larvae, affect the concomitant saprophytic carcass microbiota. To test this hypothesis, honey bee larvae were infected with a P. larvae wild type strain or P. larvae mutant strains deficient for the production of Sevadicin or Paenilamicin. Three days after infection, a mix of spores of saprophytic bacterial species was added to the larval diet. Dead larvae were analyzed by Fluorescence in situ hybridization. Our results show that only the wildtype strain of P. larvae was able to establish a pure culture of P. larvae in dead larvae, while the mutant strains were no longer able to suppress the growth of the saprophytic competitors, which instead suppressed P. larvae. Therefore, the necrotrophic lifestyle of P. larvae and the secondary metabolites are important factors for a successful spore-to-spore infection cycle.

POSTER SESSION **F-P1**

Evaluation of the toxicity and mode of action of novel fungal lectins and protease inhibitors against *Drosophila suzukii* (Diptera: Drosophilidae)

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The spotted-wing drosophila *Drosophila suzukii* (Matsumura, 1931) poses a major challenge for fruit growers worldwide. This invasive pest targets a variety of soft-skinned fruits and lays eggs in the ripening fruit. Its rapid reproduction and ability to infest undamaged fruit makes it particularly troublesome and requires innovative strategies to effectively control the pest. Our research aimed to screen selected fungal proteins against *D. suzukii*, as entomotoxic proteins found in fungi have immense potential for sustainable pest control. For this purpose, we placed fresh *D. suzukii* eggs on artificial food containing selected fungal lectins and protease inhibitors and monitored the mortality and development of pupae (16 proteins tested) and imagos (14 proteins tested). Of all proteins tested, only 4 significantly increased mortality of pupae and imagos, 9 or 18 days after treatment, respectively. AAGx1 and SSAy1 were found to be the most toxic to *D. suzukii*, with AAGx1 causing 100 % mortality of pupae and imagos and SSAy1 causing 98.7 % mortality of pupae and 95 % mortality of imagos. The EC_{50} value for AAGx1 was identified at 0.096 mg/ml (95% CI [0.083, 0.109]) for pupae and 0.126 mg/ml (95% CI [0.093, 0.147]) for imagos and for SSAy1 at 0.261 mg/ml (95% CI [0.241, 0.286]) for pupae and 0.281 mg/ml (95% CI [0.255, 0.309]) for imagos. AAGx1 and SSAy1 exposed larvae were also significantly smaller than control larvae, whereas no effects of AAGx1 and SSAy1 on the level of histological structure of the midgut epithelium were detected 3 and 6 days after treatment. Since the presence of sugars can inhibit proteins by various mechanisms, we also tested the potential inhibitory effect for AAGx1 and SSAy1 in the same experimental setup. Galactose successfully detoxified SSAy1. However, contrary to expectations, lactose did not detoxify AAGx1, but increased its toxicity 18 days after treatment by 38 % in pupae and 75.5 % in imagos, compared to AAGx1. Selected fungal proteins therefore have the potential to be a promising and interesting tool for sustainable pest control solutions.

POSTER SESSION **F-P2**

Natural incidence of *Beauveria bassiana* as biological control agent of tarnished plan bug and stink bugs in Mississippi Delta

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The stink bug complex (Heteroptera: Pentatomidae) and the tarnished plan bug (TPB) *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae) are invasive insects responsible of reducing production in soybean and cotton worldwide. Losses may vary depending on their population levels, efficacy of insecticides, and environmental conditions. Globally, is well known the high resistance of these pests to pyrethroids and organophosphates; yet chemical control remain the primary tool to manage these insects in most of southern states of U.S., and tropical, and subtropical zones in five continents. The entomopathogenic fungi *B. Bassiana* is well recognized as an essential source of myco-pesticides and is one of the most common pathogens that occur naturally that could contribute to build a sustainable environment. *B. bassiana* could be considered a potential non-chemical approach to be adopted for use in an integrated management of the tarnished plant bug or stink bug complex. Over 14,000 adults of TPB and 20,000 nymphs and adults of the stink bug complex were collected in 12 counties of MS Delta. Natural infection of *Beauveria bassiana* ranged from 2 to 33% for TPB adults. The natural infection of *B. bassiana* on stink bug populations reached >7% for *Oebalus pugnax* (Fabricius), >4% for *Nezara viridula* (L) and *Piezodorus guildinii* (Westwood) mainly on 4th and 5th instars. Low levels of natural infection (<1%) was found for and *Euschistus servus* (Say), *Chinavia hilaris* (Say), *Thyanta custator* (Fabricius), and *Podisus maculiventris* (Say). The highest incidence of *B. bassianna* was found in Yazoo county, where kudzu plants are growing on the edges of fields and on border of water canals. In general, the seasonal distribution of *B. bassiana* mainly tends to be high from April to August and coincides with the highest population level of TPB and July to September for the stink bug complex.

POSTER SESSION **F-P3**

Transcriptome analysis of *Beauveria bassiana* infected coffee berry borer *Hypothenemus hampei* (Ferrari)

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Coffee berry borer (CBB), *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae), is the notable insect pest of coffee industry all over the world. *Beauveria bassiana* (*Bb*) is widely utilized as a biological control agent to manage CBB. However, there remains a scarcity of data regarding the molecular factors that contribute to the virulence of *B. bassiana* against CBB, as well as the mechanisms underlying CBB responses to fungal infection. In this study, we performed transcriptome analysis to identify differentially expressed genes (DEGs) of both CBB and a fungal isolate *B. bassiana* -NCHU-271 during fungal infection to uncover the interaction between CBB and *B. bassiana*. Based on the results of CBB infected with *B. bassiana*-NCHU-271 compared with non-infected CBB, it was assumed that infection activates energy production by upregulated malate dehydrogenase and pyruvate kinase, besides, immune response was induced by upregulating *PGRP-LC* and *imd* genes in the IMD pathway. Regarding the fungal response, it was assumed that the fungi would activate the alanine, aspartate and glutamate metabolism, which are related to sporulation, to better infect the host. These DEGs will be further validated by qRT-PCR to confirm the outcomes of the analysis.

POSTER SESSION **F-P4**

Characterization of *Beauveria bassiana* 331R to control the two-spotted spider mite, *Tetranychus urticae*

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Microbial pesticides are harmless to the environment and have no negative effects on humans and livestock. It also has unique and diverse insecticidal mechanisms specific to insects. Among these microbial pesticide ingredients, entomopathogenic fungi have high infectiousness due to conidia with light and dispersible characteristics. The two-spotted spider mite (*Tetranychus urticae*) harms more than 200 species of plants worldwide and is difficult to detect in the early stages because it mainly attacks the underside of leaves. Also, due to the sucking pest, it is not easy to treat with chemical pesticides, making control difficult. Currently, two-spotted spider mites are controlled with chemical acaricides, but the effectiveness is gradually decreasing due to the development of

resistance, and new control methods are needed due to problems such as residues on humans, livestock, and the environment. In this study, *Beauveria bassiana* was used as a representative insecticide material using entomopathogenic fungi, and various characteristics of the 331R strain isolated from soil in Korea were analyzed. *B. bassiana* 331R (Bb331R) showed the highest growth when cultured using GY medium at pH 5.6 and 25°C. Millet medium was the best grain medium when considering conidial yield, pathogenicity, thermal stability, and UV-B stability. Aerial conidia (AC), blastospores (BS), and fungal culture filtrate (FCF) of Bb331R all showed a mortality rate of over 70% against two-spotted spider mites. Additionally, when FCF and BS were mixed, a very high mortality rate of 98% was observed. The genome size of Bb331R was approximately 36.6 Mbp and the number of genes was approximately 9,622, and it was confirmed to be a unique strain through comparison with the reference strain, *B. bassiana* ARSEF2860 strain.

POSTER SESSION **F-P5**

Storage stability of encapsulated *Batkoa* sp. spores dried in a fluidized bed

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Formulating bioproducts is crucial yet challenging, requiring control of fungal metabolic activity. The encapsulation of fungi using a carrier is an approach that has been steadily gaining consideration. The Entomophthoralean fungus *Batkoa* sp. has a great potential for pest control and is frequently found causing epizootics in sugarcane fields in Brazil. This study evaluated storage stability of submerged Batkoa propagules encapsulated in calcium alginate, washed with Milli-Q® water or sucrose, and dried in a fluidized bed. Submerged propagule (SP) production was carried out in the culture medium composed of anhydrous glucose (81 g/L) and yeast extract (3.64 g/L) in addition to salts. For the encapsulation, 4% sodium alginate, previously dissolved in ultrapure water, was added in a suspension with 10% kaolin (w/w) and SP of *Batkoa* sp. at a concentration of 10%. The granules were formed by dropping the suspension into a 0.1M $CaCl_2$ solution, and then washing with Milli-Q® water or sucrose. The granules were dried in a fluidized bed at a constant temperature of 40°C until they reached a water activity below 0.2. Viability was assessed post-drying and after storage at 4°C and 28°C for 7-45 days. Results showed comparable viability for Milli-Q Water or 4% Sucrose treatments, especially with kaolin. At 4°C, kaolin treatments stabilized at >76%, while alginate-only dropped to 43-47%. At 28°C, viability decreased notably after 30 days to 47-68%.This study is a pioneer in the investigation of formulation by encapsulation and bed drying of the fungus *Batkoa* sp. and highlighted the tolerance of encapsulated submerged propagules to the drying process.

POSTER SESSION **F-P6**

***Beauveria caledonica:* Microsclerotia formation and virulence against two lepidopteran species**

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The entomopathogenic fungus *Beauveria caledonica* has shown biocontrol activity against several pests and diseases and also has shown its ability to produce interesting bioactive metabolites. Microsclerotia (MS) are resistant structure considered as an excellent alternative to replace conidia as active ingredient in biopesticides due to the ability to well tolerate stress abiotic factors and the capacity to germinate and produce infective conidia under suitable conditions. In this context, the present work evaluated the virulence of the New Zealand strain of *Beauveria caledonica* F527 against two lepidoptera species and established the conditions to force the fungus to form microsclerotia in liquid fermentation. Four culture media were assessed. During the first four days of fermentation, the fungus produced free mycelia without the formation of defined structures. Only in the medium with C/N ratio 50:1, mycelia aggregates were observed after 8 days of fermentation and MS were observed after 10 days. *B. caledonica* reached a yield of 47 MS/mL and a biomass production of 12.13 g/L. The myceliogenic germination of produced MS was 100% and conidia production was $6{,}40\times10^5$ MS/conidia. Regarding virulence, *B. caledonica* conidia used at a concentration of $1x10^7$ con/mL produced a mortality of 55% on *P. xylostella* larvae and of 40% on *S. frugiperda* larvae. Even the strain F527 was pathogenic to both lepidopteran insects, the virulence was low. These results suggest the need to further investigate the host range of this strain that possibly is more active against insects from the order Coleoptera as it has been demonstrated for other isolates of this fungal species. From the best of our knowledge this is the first report of MS formation by *B. caledonia,* but fermentation conditions developed in this work need optimization to maximize the yield, which together with the study of other fungal traits, could underpin the development of novel bioproducts based on this fungus.

POSTER SESSION **F-P7-STU**

Fungal formulations against *Drosophila suzukii*, related drosophilids, without detrimental effects on *Apis mellifera*

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The drosophilids *Drosophila suzukii*, *D. melanogaster* and *Zaprionus indianus*, are an important pests of berry fields in Mexico. The use of attract-and-infect strategies involving entomopathogenic fungi are currently being evaluated to determine their efficiency in pest control given their favorable ecotoxicological profile compared to most synthetic pesticides. Here, we screened the attachment of conidia, pathogenicity, and virulence of 15 commercial dry powder fungal formulations (*Beauveria bassiana*, *Cordyceps javanica*, *C. fumosorosea*, *Lecanicillium lecanii*, *Metarhizium anisopliae*,*Purpureocillium lilacinum*, and *Trichoderma harzianum)*, against adults of *Drosophila suzukii.* The most virulent entomopathogenic formulations were then evaluated against *D. melanogaster*, *Zaprionus indianus*, and the honeybee, *Apis mellifera*. *Cordiceps javanica* (BeneIsari®) and *Metarhizum anisopliae* (Meta-SIN®) were pathogenic (92-95% mortality) and virulent products (median survival time: 3.5-6.0 days). These two products had

also the potential to control *D. melanogaster* (86-93% mortality, mean survival time: 4-7 d) and *Z. indianus* (72-86% mortality, mean survival time: 4-7 d) but caused low mortality of honeybees (<30%). These two formulations could be a useful alternative for the management of these pests in berry crops with an attract-and-infect approach.

POSTER SESSION **F-P8-STU**

Medium optimization and bioefficacy of *Hirsutella thompsonii* microsclerotia and blastospores for biological control of mites and ticks

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Entomopathogenic fungi represent an eco-friendly alternative to pesticides and offer a viable option for biological control. Despite this potential, little is known about the submerged fermentation of the ascomycete fungus *Hirsutella thompsonii*, which is virulent to various species of mites. In this study, we propose to investigate strategies for the production obtained through submerged fermentation with bioactivity against *Tetranychus urticae* (two-spotted spider mite) and *Rhipicephalus microplus* (cattle tick). The medium was optimized by selecting carbon and nitrogen sources, adjusting pH, and rotation to achieve high blastospore concentrations. Bioactivity was evaluated through bioassays using Petri dishes lined with moist cotton. Bean leaf discs were placed in the center of the plates to accommodate mites and ticks, which were treated with three different structures: microsclerotia (MS), blastospores (BL), and aerial conidia (CA). Each treatment consisted of ten repetitions with ten individuals each and a control for each pest studied. For BL and CA, the suspension was prepared at a concentration of 5 x 107 BL or CA/mL and sprayed directly onto the animals using a Potter tower. For MS, a concentration of 5 x 104 MS/mL per leaf was inoculated, and after 48 hours (MS48) and 72 hours (MS72), the mites and ticks were released onto the plates. The control treatment consisted of spraying a suspension of distilled water and Tween 80. The BL production was optimized with Corn Steep Liquor (nitrogen) and Maltose (carbon). The bioefficacy of *H. thompsonii* in controlling *T. urticae* was highest for CA and MS48 (65%), followed by BP (60%) and MS72 (55%). In *R. microplus*, blastospores stood out with 60% efficacy, while the other structures resulted in 55%. These results demonstrate the potential of establishing a submerged production of blastospores and the use of *H. thompsonii* as an indirect control strategy, as there is no direct spray application on the targets.

POSTER SESSION **F-P9**

The MICOTI project: towards a fungal-based integrated pest management against *Scaphoideus titanus.*

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The leafhopper *Scaphoideus titanus* is the main vector of the EU quarantine pest "*Candidatus Phytoplasma vitis*", which is associated with the *Flavescence dorée* (FD) disease in grapevine.
Due to the epidemic nature of this disease and the severity of the damage caused in vineyards, winegrowers are likely to respond with more intense insecticidal treatments. However, insecticides have a considerable economic impact on viticulture, as well as harmful consequences for human and animal health and for the biodiversity of the ecosystems. For this reason, recent European regulations insist on the urgency of minimizing the use of chemicals.
As a significant step in this direction, the project aims at developing two complementary sustainable approaches for the biocontrol of FD. Both are based on insect-fungal interactions: one explores the potential application of naturally occurring entomopathogenic fungi (EPF) against the insect, whereas the other is based on the possibility of using RNA interference (RNAi) mechanisms against the yeast-like endosymbiont of *S. titanus* (StYLS), which is phylogenetically related to entomoparasitic *Ophiocordyceps* fungi.
EPF strains will first be selected based on their pathogenicity through direct contact with *S. titanus* cuticle and, then, by their ability to spread and persist in the vegetative tissues of grapevine. The project will also assess whether EPF in the endophytic state can have detrimental effects on *S. titanus* by altering the plant metabolism.
Through genomic and proteomic characterization of StYLS, the project will identify and silence the fungal genes involved in the metabolic pathways (e.g. biosynthesis of amino acids and vitamins) considered essential for the insect survival. In this way, MICOTI will explore for the first time the possibility to interfere with the gene expression of a fungal endosymbiont to perturb the fitness of its insect host.

POSTER SESSION **MC-P1-STU**

In search of entomopathogens of the oak processionary moth in Germany

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The oak processionary moth (OPM), *Thaumetopoea processionea* (L.), is a widespread forest pest in Europe that feeds on the leaves of oak trees. The urticating, venomous setae, which develop from the third to sixth instar larvae, can cause severe allergic reactions on skin contact and pose a health risk to humans. Moreover, global climate change not only increases the abioctic stress in the forest ecosystems, but also favours the growth and continues distribution of OPM populations. To date, the biological control of OPM has been achieved through the application of commercial preparations of the *Bacillus thuringiensis* spp. *kurstaki* (Btk). However, this non-specific targeted pest control microorganism also brought negative effects to the diversity of other populations in the same niche. On the other hand, there is a lack of comprehensive information on the occurrence of other entomopathogens, certainly because the studies of OPM are hampered by the hazard posed by the larval setae. The *AntiEPS* project was launched in 2023 to close the knowledge gap on entomopathogens of OPM. In this part of the project, we aim to study the diversity of entomopathogenic bacteria, fungi and viruses, as well as parasitoids and predators of OPM across Germany. Larvae from different locations in Germany are collected and subjected to molecular and microscopic studies to identify the pathogens of OPM. This project will gain new insight into the occurrence of pathogens in OPM populations and will assist in developing an improved control strategy for this forest pest.

POSTER SESSION **MC-P2**

Investigations on the efficiency of microbial control agents, natural substances and copper compounds for the control of *Halyomorpha halys*

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The brown marmorated stink bug, *Halyomorpha halys*, is a polyphagous invasive species and a major pest of stone and pome fruit in Italy. Insecticide use is currently one of the main measures to contain *H. halys* damage in integrated apple orchards. The use of broad-spectrum insecticides is associated with negative effects on the environment and also poses a potential risk to beneficial insects, which contribute to natural pest regulation. The search for effective biocontrol agents is important to promote biological control methods. Investigations on the efficiency of alternative (potentially symbiont-targeting) substances, natural compounds and microbial control agents against *H. halys* were carried out under laboratory and semi-field conditions. A laboratory screening on egg masses was performed in order to evaluate the ovo-larvicidal activity of different natural substances, copper compounds and entomopathogenic fungi. The study examined i.a. substances that have been reported to negatively affect survival by disturbing the vertical transmission of the primarily bacterial symbiont, Candidatus *Pantoea Carbekii*, from female adults (via egg smearing) to aposymbiotic neonates.
Different commercialized mycopesticides and EPF strains isolated from field-collected insects or obtained from culture collections were tested for their efficiency against laboratory reared and field collected imagines by immersion bioassays.
First-instar nymphs were highly susceptible to *Metarhizium* spp. and *Beauveria* spp. under constant laboratory conditions. Under variable semi-field conditions, no or varying ovo-larvicidal efficacy of mycopesticides was observed in different trials and setups. Copper containing fungicides, a copper-zinc micronutrient fertilizer and a bacterial biocontrol product (*Bacillus amyloliquefaciens*) had no significant effect on ovo-larval mortality in any of the performed experiments.

POSTER SESSION **MC-P3**

Novel biopesticide solutions for UK pests and diseases, introducing two UKRI projects

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This poster introduces two projects funded by UK Research and Innovation (UKRI) which are both looking at novel, biological solutions for some key pests and diseases in UK arable farming. The two projects are both in their first year and bring together partners that span biopesticide research, product commercialisation and farmer advisory services. The first project is focussed on the English grain aphid (*Sitobion avenae*), the Bird cherry-oat aphid (*Rhopalosiphum padi*) and Septoria, in wheat. This project is looking at fungi isolated from UK-soils and investigating whether any of the fungal isolates have both insecticidal and fungicidal activity; in-effect whether a single fungus could be used as a dual-action biopesticide. The second project is looking at a number of biopesticides, both commercialised and prototype preparations, to build a suite of near 'ready-to-go' controls for use in UK legumes. This project is targeting the Pea and bean weevil (*Sitona lineatus*) and foot rot pathogens; as well as looking at growth promotion of legumes when under drought stress. The biopesticides include microorganisms and natural substances. Both projects started in the lab, screening against the target pests and diseases and have recently moved into glasshouse trials (for aphids/Septoria) and into field-settings for the legumes' project. The projects, combined, bring together CABI, the UK Agri-Tech Centre, FA-Bio, Agrii, Russell Bio, Fargro and the University of Warwick.

POSTER SESSION **MC-P4**

Bioprospecting environmental fungi of Crete for the development of mosquito biopesticides

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The increasing emergence of insecticide resistance among disease vectors, coupled with the negative impacts of chemical pesticides on humans, animals and the environment, are promoting the development and application of biopesticides worldwide. Among prospective biopesticides are entomopathogenic fungi and their secondary metabolites. To enrich the arsenal of biopesticides, we surveyed a range of diverse natural habitats in Crete and established a microbial library of over 1,500 bacterial and fungal isolates, of which 46 are fungi. Bioassays conducted against larvae of the mosquito vector *Culex pipiens molestus* resulted in a selected group of 9 isolates showing potent entomopathogenic activity. Four isolates of *Cladosporium* and one from the family Mortierellaceae (putatively *Mortierella*) showed potent activity against the larvae and have not had their potential as biological control agents fully explored. Our ongoing studies are focusing on the spectrum of activity and other characteristics of these isolates to evaluate their suitability for their development and commercialization as biopesticides for integrated vector management programs.

POSTER SESSION **MC-P5**

Using nanoparticles to enhance dsRNA uptake efficiency in *Phthorimaea absoluta*

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The South American tomato pinworm, *Phthorimaea (=Tuta) absoluta* is an invasive pest in Africa and Europe, where it causes substantial yield losses to tomato production, yet control options are highly limited. Currently, synthetic insecticides are mainly used for the control of the pest, which remains however unsustainable. The use of RNA interference (RNAi) technology as an alternative pest control strategy is gaining prominence worldwide. However, the success of this control method depends on a highly efficient and stable double-stranded RNA (dsRNA) delivery system to enhance gene silencing, especially in *P. absoluta*. In this study, we are testing the efficacy of two nanoparticles, clay nanosheet and chitosan, complexed with dsRNA, to target two *P. absoluta* genes including the *juvenile hormone inducible protein* (*JHP*) and the *chitin synthase A* (*CHI*). We will report a bioassay system allowing the use of an artificial diet for *P. absoluta* to test different formulations of dsRNA.

POSTER SESSION **MC-P6**

Environmental Constraints to *Beauveria* Efficacy for *Cimex lectularis* (Bedbug)

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In 2018 a *Beauveria bassiana* (strain GHA) was registered and commercialized in the U.S. for bedbug control. While the typical habitat for *Cimex* is more protected than that of agricultural pests, it can vary in temperature and humidity, especially in low income housing. We investigated the effect of a matrix of temperature (15, 25, 32° C.) and humidity (30, 50, and 70%) on the pathogenicity of the *Beauveria* for bedbugs, as well as on efficacy and conidial viability during 4 weeks post treatment. Equal numbers of adult and immature bedbugs were bioassayed using the commercial oil formulation applied per label-recommended method, with three replicates per condition. Groups of 20 bedbugs were exposed to the Beauveria by crawling over sprayed, latex-painted tiles for a standard time duration. Overall mortalities were 56-78% after 14 days, resulting from a conidial concentration of 3-4x10^4 conidia mm^{-2} sprayed surface. We observed little effect of temperature and humidity on pathogenicity, within the parameters of the bioassays, using Day 14 control-corrected mortality. In addition, conidial viabilities from the tiles were determined at 2 and 4 weeks post-spray. We observed a severe loss in conidial viability of 21% to 99.9% at 2 weeks depending on conditions, to < 1% at 4 weeks for all conditions except 30% humidity at 15 and 25° C., at which viabilities dropped to 27-29%. In a bioassay testing persistence in terms of efficacy, bedbug mortality after 2 weeks of weathering at 25° C and 50% humidity dropped from an initial 73% to 40% (conidial concentration 4x10^4 conidia mm^{-2} sprayed surface).

POSTER SESSION **MC-P7**

Spore Persistence of *Beauveria* Wettable Powder and Emulsifiable Concentrate Formulations Under Lower Rio Grande Valley, Texas, Climate Variables

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Commercial mycoinsecticides rely on fungal entomopathogenic spores to induce infection in target pest insects. However, typical field conditions in the Lower Rio Grande Valley of Texas USA, a sub tropical plain of thorny shrubs and trees with scattered palm and woodlands, present less than ideal year around conditions for fungal efficacy. After application and before infection, fungal conidia are exposed to various environmental variables, including UV radiation, low-humidity driven desiccation, and windborne physical displacement, as well as potentially inhibitory temperatures. We studied the impact of these factors over time on the persistence of infectivity against the Asian Citrus Psyllid (*Diaphorina citri* Kuwayama), a serious citrus pest of the region as well as elsewhere, after application of select commercial mycoinsecticides to orange jasmine citrus. Two commercial *Beauveria bassiana* products were evaluated at high recommended label concentrations: a wettable powder (Bioceres® WP) and an emulsifiable concentrate (Bioceres® EC) to determine persistence of conidial viability after different amounts of natural levels of solar radiation, humidity and temperature. Conidial viability in the EC remained above 50% after 7 days indoors versus dropping to 30% outdoors, while the WP showed considerably lower even < 0.1% viability in either setting. In addition, we have evidence that conidia in the WP dislodge from leaf surface over time significantly lowering the concentration, and thus numbers of conidia encountered by an immigrating psyllid.

POSTER SESSION **MC-P8**

Engineering of multiple trypsin/chymotrypsin sites in Cry3A to enhance its activity against *Monochamus alternatus* Hope larvae

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Bacillus thuringiensis Cry3 toxins exhibit specific toxicity against several coleopteran larvae. However, owing toits low toxicity to *Monochamus alternatus*, Cry3A toxin is not useful for managing *M. alternatus* larvae. Here we assessed the proteolytic activation of Cry3Aa toxin in *M.alternatus* larval midgut and increased its toxicity by molecular modification. Our results indicated that insufficient processing of Cry3Aa protoxin and non-specific enzymatic digestion of Cry3Aa toxin in the midgut of *M. alternatus* larvae led to low toxicity. The results of transcriptome analysis, enzymatic assay with fluoro-genic substrates, and multiplex substrate profiling by mass spectrometry showed that the main digestive enzymes in *M. alternatus* larval midgut were trypsin-like proteases that preferentially cleaved peptides with arginine and lysine residues. Consequently, trypsin recognition sites were introduced into the Domain I of Cry3Aa protoxin in the loop regions betweenα-helix 3 and β-helix 4 to facilitate proteolytic activation. Multiple potential trypsin cleavage sites away from the helix sheet and functional regions in Cry3A aproteins were also mutated to alanine to prevent non-specific enzymatic digestion. Bioassays indicated that a modified Cry3Aa-T toxin (K65A, K70A, K231A, K468A, andK596A) showed a 9.5-fold (LC50 = 12.3 ug/mL) increase in toxicity to *M. alternatus* larvae when compared to native Cry3Aa toxin. This study highlights an effective way to increase the toxicity of Cry3Aa toxin to *M. alternatus*, which may be suitable for managing the resistance of transgenic plants to other pests, including some of the most important pests in agriculture.

POSTER SESSION **MC-P9**

Cadherin gene is involved in the toxicity of *Bacillus thuringiensis* subsp. *aizawai* to *Spodoptera frugiperda*

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Gram-positive and spore-forming entomopathogenic bacterium, *Bacillus thuringiensis* (Bt), has been predominantly used for microbial biopesticides in the global market due to its high specifically to target pests and low impact on non-target organisms. Bt subspecies *aizawai* (Bta) and *kurstaki* (Btk) are important microbial control agents for lepidopteran insect pests. The fall armyworm (FAW), *Spodoptera frugiperda*, is regarded as one of the most destructive pests of maize. The FAW originated from the American continent, spread to Africa, and invaded Korea through China in 2019. In Bt toxicity against FAW, Bta appears to be more potent than Btk from 2nd to 4th instar larval stages. The suppression of *S. frugiperda cadherin 1* (*SfCad1*) expression in 4th instar larva significantly reduced susceptibility to Bta, but not in Btk. These results suggest that *SfCad1* is involved in a Bta toxicity.

Insights into Insects-Fungus Interplay: Transcriptomic Profiling of *Metarhizium anisopliae* Treatment

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Entomopathogenic fungi serve as eco-friendly alternatives to chemical pesticides. In this study, we investigate the interactions between insects and Metarhizium anisopliae, which showed high insecticidal activity against insects, by RNA-seq analysis. RNA from insects was extracted at the median lethal time to identify changes in gene expression. The results showed 580 genes were up-regulated, while 336 genes were down-regulated in fungal treated insects. Up-regulated genes were related to metabolic and cellular processes such as cytochrome P450, DNA replication, and apoptosis. Down-regulated genes were involved in metabolism pathways such as lysosome, starch and sucrose metabolism, and fatty acid biosynthesis. Within insects, 948 genes of fungi have been identified, with key genes related to energy and protein metabolism such as ribosome, oxidative phosphorylation, citrate cycle, glycolysis/gluconeogenesis. Additionally, genes influencing apoptosis and DNA repair and damage prevention pathways in response to stress factors have been identified. These results are crucial for elucidating the mechanisms of fungal invasion and interaction in insects, providing insights for future pest management strategies.

Proteomics for studying the microbiome in non-model insects

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Many plant diseases are caused by viruses and bacteria transmitted by insect vectors that can spread these pathogens to a wide range of species of agricultural and forestry interest. All insects live in close association with a community of microorganisms, collectively known as the microbiome, that colonise their body and can influence their physiology, ecology and evolution. Microbiomes can also alter the vector competence of their insect hosts, affecting their ability to acquire or transmit the pathogen. Therefore, an in-depth understanding of the microbiome can lead to the development of new strategies for sustainable pest control.
In this work we will use recent technological advances in the field of metaproteomics to characterise the interactions between the leafhopper *Euscelidius variegatus* (Hemiptera: Cicadellidae) and its microbiome. *E. variegatus* is, under laboratory conditions, the vector of the phytoplasma associated to Flavescence dorée (FDp) a grapevine disease that threatens the production of European vineyards. Two LC-MS/MS-based quantitative approaches will be used: the classical data-dependent acquisition (DDA) and the data-independent acquisition (DIA), which ensures the identification of proteins in complex mixtures even at low concentrations. A tailored sequence database will be built by integrating all available genomic information about the insect and its microbiome, in order to identify as many proteins as possible and the main active metabolic pathways.
The final objectives of this study are i) to identify the proteins expressed by the *E. variegatus* microbiome; ii) to establish guidelines for metaproteomics experiments on insect microbiomes that can be applied to different species, especially non-model species for which genomic data are scarce.

Effect of beneficial microbes on aphid development and predatory hoverflies host choice-selection

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Aphids are prime pests of several crops but the environmental risks associated with their chemical control pose an urgent need to create a sustainable management. Plant growth-promoting rhizobacteria (PGPR) and hypocrealean entomopathogenic fungi (HEF) are beneficial microbes that can enhance plant growth traits and increase resistance to abiotic and biotic stressors. Whereas the role of PGPR and HEF in plant protection in agriculture has been reported, there is little information about their impact on trophic interactions including those between pests and their predator fauna. Here, we characterize the effect of the two PGPR *Bacillus velezensis* GA1 and *Pseudomonas* CMR12a, and the two HEF *Metarhizium brunneum* GxABT-2 and *Metarhizium brunneum* U4556 to colonize tomato plants, on fitness of the potato aphid *Macrosiphum euphorbiae* (Thomas, 1878) and the aphidophagous hoverfly, *Episyrphus balteatus* (De Geer 1776). Through a series of life table analyses, we found that *M. euphorbiae* was susceptible to PGPR/HEF-treated tomato. Also, the treated tomato significantly impacted the plant choice and oviposition behavior of *E. balteatus*. These results are of utmost interest since they help to select the most suitable microbial agents against aphids without harming beneficial insects.

Ultrastructure of *Tubulinosema loxostegi* Malysh Tokarev Issi 2013 re-isolated from West Siberian populations of beet webworms and propagated in Siberian silkmoth *Dendrolimus sibiricus*

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Microsporidia of the genus *Tubulinosema* are characterized by extreme host plasticity and may represent a threat to human health causing fatal infections in immunosuppressed patients. *Tubulinosema loxostegi* was described in 2013 basing on SSUrDNA characterization and the ultrastructure of spores isolated from dry cadavers of *Loxostege sticticalis* adults collected in West Siberia. The microsporidium was re-isolated in 2020, and since then was propagated in laboratory cultures of various lepidopterans including Siberian silkmoths *Dendrolimus sibiricus*. Small Subunit rDNA sequence analysis of the 2020 isolate of *T. loxostegi* indicted 99.7% similarity to the 2013 isolate. The pairwise similarity between *Tubulinosema* species varies from 99.3 to 99.6%. Thus, to clarify whether the novel isolate is in fact *T. loxostegi,* and to obtain more information on parasite-host interactions, TEM analysis was undertaken. Our study demonstrated spore dimensions and ultrastructure like those previously described (Malysh et al., 2013), namely: (1) spores 3.6-4.5 x 2.0-2.6 µm, (2) spore walls with thick endospore and multilayered exospore, (3) bipartite lamellar polaroplast, (3) slightly anisofilar polar tube of 10-12 coils, and (4) an indentation of the spore wall at the apical region. In addition to the

previous study, pre-spore stages were characterized. All of them were diplokaryotic and developed without interfacial envelopes. Late meronts bared the layer of fine tubules on their plasma membrane, the apomorphic trait of *Tubulinosema* spp. The microsporidium was found exclusively in Malpighian tubes and caused hypertrophy of this organ. Genomic study of both *T. loxostegi* isolates from laboratory and native hosts is underway and will potentially explain high levels of interspecies SSUrDNA sequence divergence.

POSTER SESSION **MS-P2**

Olfactory recognition of microsporidia-infected *Drosophila suzukii* by parasitic wasps has consequences for wasps´ fitness

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Microsporidian infection in *Drosophila suzukii* (spotted wing drosophila, SWD) with *Tubulinosema suzukii* diminishes eclosion rates, survival, and lifetime fecundity of the fly under laboratory conditions. Here, we examined whether *T. suzukii* infection of its primary host also negatively impact parasitisation success and eclosion rates of parasitic wasps *Pachycrepoideus vindemiae*, *Trichopria drosophilae* and *Spalangia erythromera* which are considered natural antagonists of SWD. Our data revealed *T. suzukii* infection in host (SWD) pupae only affected *T. drosophilae*, with significantly reduced eclosion rates and transmission rate by 30% from host to wasp progeny. For *P. vindemiae* and *S. erythromera*, parasitisation and eclosion rates did not significantly differ compared to controls suggesting a negligible effect on the development cycle of these species. Two species, *T. drosophilae* and *P. vindemiae*, were unable to detect infected host pupae in choice test allowing a direct visible, haptic, and odorous contact to the host pupa, and in olfactometer experiments examining only the odorous component. *S. erythromera* was the only parasitoid able to distinguish infected hosts, preferring the healthy pupa in olfactometer although this species was not susceptible to *T. suzukii* transmission. Consequently, *T. drosophiliae* seems the only species susceptible and vulnerable to *T. suzukii* transmission from infected host with yet unknown consequences for laboratory rearing or natural populations faced with microsporidian pathogens.

POSTER SESSION **MS-P3-STU**

Effect of the chitosan-based dsRNA nanocomplex on microsporidian parasites *Nosema ceranae* in the honey bee

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Honey bees are one of the important insects in the world as pollinators of numerous agricultural crops. Honey bees have faced many diseases which threaten bee colony including a serious population decline phenomenon called Colony Collapse Disorder (CCD). *Nosema ceranae* is a pathogen cause nosemosis, also called Nosema, which is the one of most common disease of bees. According to the genome sequencing for *N. ceranae*, it has been identified that the presence of machinery for RNA silencing and RNA interference technology (RNAi) has successfully silenced the ADP/ATP transporter gene in this species under laboratory conditions. Accordingly, we have already reported that the proliferation of Nosema can be effectively inhibited by treatment of dsRNA for FNR2 gene. However, various limitations that need to be resolved for the bio-application of dsRNA have already been reported, among them the low delivery stability of dsRNA is considered the most important problem. Therefore, in this study, we aimed to improve the delivery efficiency of dsRNA for FNR2 in the honey bee. For this purpose, dsRNA nanocomplex was formed using chitosan to evaluate the enhancement of RNAi treatment effect. In addition to confirming the formation of the nanocomplex, the safety of the dsRNA-chitosan nanocomplex for honey bees was evaluated to first look at the possibility of evaluating its effectiveness. In addition, the production of Nosema spores and the survival rate of honey bees were evaluated according to the treatment interval of the dsRNA-nanocomplex. As a result, it was confirmed that the effect of RNAi can be increased through dsRNA nanocomplex treatment using chitosan.

POSTER SESSION **MS-P4**

Analysis of the genetic diversity of the honey bee (*Apis mellifera*) pathogen *Nosema* spp. based on Short Tandem Repeats (STR)

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Nosema apis and *Nosema ceranae* are microsporidian parasites of the Western honey bee (*Apis mellifera*), which infect the epithelial cells of the midgut of adult bees. While *N. apis* is known as a pathogen specific for *A. mellifera* since more than 100 years, *N. ceranae* was originally described as a pathogen of the Eastern honey bee *A. cerana*, but obviously switched host several decades ago and by now is even more prevalent than *N. apis* in many *A. mellifera* populations. Results on the differences in virulence between *N. ceranae* and *N. apis* are contradictory as are reports on the replacement of *N. apis* through *N. ceranae* in *A. mellifera* populations. One possible explanation for these contradictory results is the described sensitivity of *N. ceranae* spores to cold, which could have a climate-dependent effect on the spread and virulence of *N. ceranae*. It was also suggested, that assertiveness and virulence differ between different populations of *N. apis* and *N. ceranae*, which would indicate a population genetic effect in the two species. Several studies attempted to characterize *Nosema* spp. on a population genetics level in order to investigate the genetic differentiation between phenotypically different or geographically distant *N. ceranae*- or *N. apis*-populations. While the results for *N. ceranae* are contradictory, there is evidence for genetic variance between *N. apis*-isolates from geographically distinct *A. mellifera* samples. This prompted us to develop a short tandem repeat (STR) -based analysis adapted to the characterization of the genetic diversity of *N. apis*. We tested 60 pairs of primers flanking putative STR-regions of the *N. apis* genome and after evaluation and optimization, five pairs of primers were identified which were suitable to identify genetic variants. We present our data from the STR-based analysis of the *N. apis* population circulating in the *A. mellifera* population in northeastern Germany.

POSTER SESSION **MS-P5**

Genomic variations among *Vairimorpha ceranae* different isolates revealing microsporidia might adapt to thermal stress in Taiwan

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Vairimorpha ceranae is the pathogen responsible for microsporidiosis in *Apis mellifera*, a disease characterized by absolute intracellular parasitism, leading to physiological and behavioral adversities resulting in honey bee death. Our monitoring data suggest that mature spore counts increased as temperatures increased in *A. mellifera* colonies in 2020-2021 in Taichung, Taiwan, which was different from the previous report in 2012. To better understand if there are variations among different *V. ceranae* isolates, which might contribute to the host of environmental stresses, we aim to identify potential different genomic regions that may contribute to changes in the adaptability of *V. ceranae* in Taiwan. In this study, we employed Oxford Nanopore Technology (ONT) to re-sequence the genome of *V. ceranae* in the honey bee population in Taichung, Taiwan (Vc-Tw). We compared it with three published *V. ceranae* genomes. The genome size of Vc-Tw is 7.9 Mbp, including 88 contigs. The comparative genomic data identified 32 distinctive genomic regions (DGRs) with identification rate < 75% and length > 150 bp from published *V. ceranae* genomes. Among these DGRs, four genes, including a*purinic/apyrimidinic endonuclease* (*AP endonuclease*, AAJ76_138000217), *16S ribosomal RNA* (AAJ76_1700061205), *23S ribosomal RNA* (AAJ76_1700062503) and hypothetical protein (AAJ76_3000167792), were identified and showed differential expression levels between various stages of *V. ceranae* infection based on previous transcriptome data. The *AP endonuclease* was only expressed at mature spore, 16S and 23S ribosomal RNA expressed at mature spore, 5 ,10 and 20 days point infection (dpi), hypothetical protein AAJ76_3000167792 was highly expressed at 10 and 20 dpi., suggesting these genes might involve in the adaptability of *V. ceranae*. Further investigations will focus on the four differential expressed genes in the DGRs to evaluate the implications of genomic variations on *V. ceranae* and the connection to environmental adaptation.

POSTER SESSION **N-P1**

Resolution of efficiency of entomopathogenic nematode *Steinernema germanica* sp. (Rhabditida: Steinernematidae) on the potato tuber moth (*Phthorimaea operculella* (Zeller)) (Lepidoptera: Gelechiidae) under laboratory conditions

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The potato tuber moth, Phthorimaea operculella (Zeller) (Lepidoptera: Gelechiidae), is an important pest of potato that causes yield loses in potato producing part of Georgia in region of Samtskhe-Javakheti. Many management methods exist to reduce the population of this pest. Chemical control methods are in the first place in Georgia. However, entomopathogenic nematodes (EPNs) can be used as a potential alternative to chemical insecticides to control potato tuber moth larvae as an environmentally friendly management method. We aimed in this study was to examine the efficacy of isolates of Steinernema germanica sp. against the last instar larvae (fourth stage) of the potato tuber moth under laboratory conditions. Experiments were carried out in 150 milliliter plastic cups with a sterile soil mixture. Four concentrations of entomopathogenic nematodes (0, 300, 600 and 900 IJs) were applied directly to the soil. Potato tuber moth was sensitive to different concentrations of Steinernema germanica sp. All doses of Steinernema germanica sp were more effective than the control (water). The effective was the concentration of 900 IJs, which provides a high mortality rate of the last instar larvae of potato tuber moth. No statistically significant difference was observed among temperatures. Our results showed that the isolate of the entomopathogenic nematode Steinernema germanica sp is effective and can be used for biological control against potato tuber moth, Phthorimaea operculella.

POSTER SESSION **V-P1**

Genomic analysis of different American strains of SfNPV baculovirus isolated from *Spodoptera frugiperda*

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Spodoptera frugiperda is the main pest of corn due to its economic impact. The use of baculovirus isolated from this insect (SfNPV) represents an alternative to chemical insecticides. In our work group, some strains of SfNPV from different geographical points in America have been previously isolated and characterized. The most virulent strain was isolated from Argentina (SfNPV-Ar) and the least virulent isolated in Mexico (SfNPV-Sin), with the strain isolated from the United States (SfNPV-Fx) presenting intermediate levels. These strains were sequenced, annotated and their genomes analyzed, and compared with strains such as SfMNPV isolates 281, ColA, 459, NicB, 1197, 19, 3AP2 and NicG. Genome sizes of 134,254 bp for SfNPV-Sin, 134,250 bp for SfNPV-Fx and 134,239 bp for SfNPV-Ar were obtained; 146 and 147 open reading frames (ORFs), respectively. Aligning their genomes, were found identity percentages greater than 99.5% with respect to SfNPV-19 (97% coverage) and percentages less than 98% with SfNPV-459 (100% coverage). Regarding strain 459, were found an average of 930 nucleotide changes, 69 insertions (1-19 nucleotides) and 46 deletions (1-17 nucleotides). For the strain 19, were observed an average of 95 nucleotide changes, 4 insertions (1-6 nucleotides) and 5 deletions of between 1 to 26 nucleotides. These changes modified the triplets of the ORFs, so the percentages of identity for the amino acid sequences, with respect to the previously ORFs reported, were determined by multiple alignments. LEF-7 presented identity percentages (ID) of 98.4-100%. Structural proteins of budded virions and occlusion-derived virions, such as, ME53 (98.3-100% ID), GP16 (98.9-100% ID), LEF-2 (97.6-100% ID), PKIP-1 (98.3 -100% ID), GP41 (98.2-100%ID), 38.7K (97.3-100% ID), P78 (98.2-100%), P40 (98.9-100), SOD (98.6-100% ID), VP80 (98.2-100% ID), CG30 (98-100% ID), GP37 (94.3-99.6) and VP91 (90-99.8% ID). Finally, the EGT protein presented 96-100%. In the AcMNPV and BmNPV model baculoviruses, it has been determined that these types of changes may affect *in vitro* and *in vivo* infection. Due to the above, this work lays the bases to try to understand if these differences found in these specific genes between the 3 study SfNPV strains, are a key factor that explains the different levels of virulence reported between them.

POSTER SESSION **V-P2**

Identification of the molecular basis of CpGV resistance in codling moth, *Cydia pomonella*

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The larvae of *Cydia pomonella* (codling moth, CM), bore into unripe apples and complete their development inside, results in non-marketable fruits and high economic loss. Different Cydia pomonella granulovirus (CpGV) preparations including resistance-breaking CpGV isolates have been applied to reduce infestation and avoid fruit damage caused by susceptible and resistant (Type I-III) codling moth populations in pome fruit production in both organic and integrated pest management (IPM) plantations. The Type I resistance of CpGV to codling moth were found in seven European countries and in Washington State (US), which requires to further explain the mechanism of the widely occurred Type I resistance in codling moth.
The present project is aiming to elucidate the early event in CpGV infection process in codling moth. To study the role of *pe38* in CpGV infection and resistance process of CM larvae, stably transformed Cp14R-pe38 cell line is established using *Sleeping Beauty* transposon system. Antibiotic marker of *Zeocin* and florescent marker of *mCherry2* are fused at C terminal of *pe38* in order to select the corrected Cp14R-pe38 cells. Consistent expressed PE38 and its complex are collected in pull-down assays. LC-MS/MS and NGS sequencing are applied to determine the sequence of amino acid derived from PE38 complex and nucleotide sequences anchored, respectively. The PE38-targeted gene/sequence in either host chromosome or viral genome can be identified.
The final outcome can answer the basic question how the codling moth blocks CpGV replication in early stage of infection and how to reduce/eliminate type I resistance and additional resistance cases by disrupting this infection pathway. Elucidating the mechanism of CpGV resistance to codling moth can not only figure out a scientific issue, but also point out the way for sustainability of baculovirus formulation in pome fruit production.

POSTER SESSION **V-P3**

The genomes of an alphabaculovirus and a betabaculovirus from the tufted apple bud moth, *Platynota idaeusalis*, reveal recent recombination between baculoviruses that share a host

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The complete genome sequence of a betabaculovirus from the tufted apple bud moth, *Platynota idaeusalis*, was determined from an occlusion body sample deposited in the USDA insect virus collection at Beltsville, Maryland in 1981. The sequence of this virus, Platynota idaeusalis granulovirus isolate 2683 (PlidGV-2683) indicated that the genome was a circular DNA of 106,633 bp with a 31.37% G+C nucleotide distribution. Although no homologous regions (*hr*s) were detected the genome, five intergenic regions containing tandem direct repeats of different 21 – 28 bp sequences were documented. A total of 125 ORFs were annotated in the genome sequence, including nine ORFs with no homologs in other sequenced baculoviruses. Phylogenetic inference based on alignments of the baculovirus core gene amino acid sequences placed PlidGV-2683 in clade b of the betabaculovirus tree, in a node with Adoxophyes orana granulovirus with 71% bootstrap support. Comparison with the previously sequenced Platynota idaeusalis nucleopolyhedrovirus (PlidNPV-2680) revealed a 1,516-bp region in PlidNPV-2680 adjacent to a homologous region (*hr9*) that exhibited 97.5% sequence identity to PlidGV-2683 nt 4510 – 5158 and 97% sequence identity to PlidGV-2683 nt 5330 – 6189. This region suggests that recombination between PlidNPV and PlidGV occurred recently in larvae infected with both viruses, resulting in an acquisition of sequence by PlidNPV from PlidGV. The acquired region in PlidNPV contains homologs of PlidGV ORF7 (*ac146-llke*), ORF9, and ORF10 (*odv-e18*), but the *ac146-like* and *odv-e18* homologs are truncated. The acquired *odv-e18* homolog in PlidNPV-2680 is missing the N-terminal sequence involved in occlusion-derived virus envelope localization. The sequence and analysis of the genomes of these two *P. idaeusalis* baculoviruses provides an example of how baculovirus evolution can be influenced by recombination with viruses that share the same host range.

POSTER SESSION **V-P4**

Autographa californica Multiple Nucleopolyhedrovirus *ac106* Is Required for Intranuclear Microvesicle Formation, and Intranuclear Microvesicle Formation is Essential for the Intranuclear Transport of ODV Integral Envelope Proteins

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Baculovirus occlusion-derived virions (ODVs) are known to acquire their envelopes from virus-induced intranuclear microvesicles within the nucleoplasm, and this strategy of the intranuclear envelopment of nucleocapsids to form virions is unique among viruses. However, the mechanism of ODV morphogenesis, particularly intranuclear microvesicle formation, remains unclear. In this study, we identified Autographa californica multiple nucleopolyhedrovirus (AcMNPV) *orf106* (*ac106*) as the fifth gene, in addition to *ac75*, *ac76*, *ac93*, and *p48*, that is required for intranuclear microvesicle formation. Besides intranuclear microvesicle formation, *ac106* is required for the nuclear egress of nucleocapsids. It is thus involved in both budded virions (BVs) and ODV production and the embedding of ODVs into polyhedra. Ac106 is a baculovirus late protein that is concentrated in discrete foci of virus-induced membrane structures in the intranuclear ring zone of virus-infected cells. Further studies on the relationship between Ac106 and four other proteins that are also required for intranuclear microvesicle formation showed that Ac106 associated with Ac76, Ac93, P48, and itself. Ac106 is required for Ac75, Ac93, and P48 accumulation in foci of virus-induced intranuclear membrane structures and the intranuclear transport of Ac76. Analysis of the subcellular localization of ODV integral envelope proteins upon deletion of the genes required for intranuclear microvesicle formation indicated that intranuclear microvesicle formation may be essential for ODV integral envelope protein transport into the nucleus, supporting the hypothesis that intranuclear microvesicles originate from the nuclear membrane. These findings greatly enhance our understanding of the molecular mechanism of baculovirus ODV morphogenesis.

POSTER SESSION **V-P5**

Recombinant SpfrGV enhancin proteins improve the insecticidal activity of SfMNPV-ColA on *Spodoptera frugiperda* larvae

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Spodoptera frugiperda represents a significant threat to maize crops worldwide. To control this pest, the use of microbial biological control agents has been proposed. Among these, various genotypes of Group II alphabaculoviruses like SfMNPV ColA have been identified and they proved to be useful for such purposes. However, the efficacy of SfMNPV-based formulations must be improved to optimize their performance in the field. Regarding this, although SpfrGV (VG008), a betabaculovirus that infects the same lepidopteran, is not effective as a standalone bioinsecticide, its combination with SfMNPV-ColA exhibits a synergistic effect, enhancing insecticidal efficacy. Notably, the SpfrGV (VG008) genome features 4 chitin-binding proteins and 2 metalloproteases, Enhancin 1 (Enh-1) and Enhancin 2 (Enh-2), that could be related to the improved performance of SfMNPV-ColA. Based on this, Enh-1 and Enh-2 proteins were characterized bioinformatically and expressed in *Escherichia coli*. Our results show that the recombinant proteins have *in vitro* proteolytic activity on casein as enzyme substrate, because they present peptidase motifs in their sequence. Additionally, bioassays with SfMNPV-ColA were carried out in *Spodoptera frugiperda* larvae, supplementing with each of the SpfrGV-derived recombinant proteins (alone and combined). In all cases, the presence of betabaculovirus protein factors significantly improved the efficacy of SfMNPV-ColA. This opens new productive paths for the formulation of a new generation of bioinsecticides based on baculoviruses without the need to modify their genomes.

POSTER SESSION **V-P6-STU**

Increasing production efficiency of target proteins by regulating VLF-1 in baculovirus expression system

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The baculovirus expression system (BES) is used to produce useful proteins through generation of recombinant viruses. Recombinant protein production can utilize the *p10* or *polyhedrin* promoter, a very late promoter that exhibits strong transcriptional activity at late in viral infection. The burst sequence (BS) is essential for robust transcription of very late promoters. Very late expression factor 1 (VLF-1) is involved in the structural proteins of BV and ODV, but is also known as a transcription factor that specifically binds to the BS of very late promoters. It has also been reported that VLF-1 is able to regulate the amount and timing of protein expression by very late promoters. We constructed recombinant viruses expressing EGFP as a marker gene under the *p10* or *polyhedrin* promoters, while additionally expressing VLF-1 under various promoters. By evaluating the level of VLF-1 expression by various promoters and the resulting EGFP expression efficiency, the optimal VLF-1 expression conditions for activating the *p10* or *polyhedrin* promoter were determined. In addition, the expression efficiency of the target protein by the *p10* or *polyhedrin* promoter was evaluated by knocking out VLF-1 present in Bacmid and constructing recombinant Bacmid that expresses VLF-1 in situ by various promoters. As a result, it was possible to determine the optimal VLF-1 expression conditions to increase the production efficiency of the target protein.

POSTER SESSION **V-P7**

Function of AcMNPV-miR-2 in AcMNPV infection

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Virus-encoded miRNAs exert diverse regulatory roles in the biological processes of both viruses and hosts. This study delves into the functions of AcMNPV-miR-2, an early miRNA encoded by Autographa californica multiple nucleopolyhedrovirus (AcMNPV). AcMNPV-miR-2 targets viral early genes *ac28* (*lef-6*), *ac37* (*lef-11*), *ac49*, and *ac63*. Overexpression of AcMNPV-miR-2 led to reduced production of infectious budded virions (BVs) and diminished viral DNA replication. Delayed polyhedron formation was observed through light and transmission electron microscopy, and the larval lifespan extended in oral infection assays. Moreover, the mRNA expression levels of two Lepidoptera-specific immune-related proteins, Gloverin and Spod-11-tox, significantly decreased. These findings indicate that AcMNPV-miR-2 restrains viral load, reducing host immune sensitivity. This beneficial effect enables the virus to combat host defenses and reside within the host for an extended duration.

POSTER SESSION **V-P8**

Combining SIT and baculovirus application in an integrated approach to management of the false codling moth

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The false codling moth, *Thaumatotibia leucotreta*, is an important phytosanitary pest of citrus and other fruit and nut crops in sub-Saharan Africa. Due to their sustainability, non-chemical means of control are preferable. Development of the Cryptophlebia leucotreta granulovirus (CrleGV) as a biopesticide for control of *T. leucotreta*, began in in South Africa in 1998. CrleGV has now been extensively tested in the field for many years with more than 50 trials completed, and has been used commercially over tens of thousands of hectares annually for two decades, mainly on citrus crops. Trial results show that applications of CrleGV have consistently led to significant reductions in fruit infestation in relation to untreated controls. The highest reduction in infestation was reported to be 92%, while the lowest was 27%. While such variability in levels of suppression may be undesirable, this underscores the importance of an IPM programme consisting of various technologies to cumulatively reduce pest levels. Consequently, the sterile insect technique (SIT) was developed for *T. leucotreta* suppression and commercialised in 2007. Since the inception of the programme, SIT has reduced moth catches by 99%, fruit infestation by 96%, and export rejections by 89%. As the ideal area-wide approach to pest management, it provides a perfect foundation for orchard specific control measures, such as CrleGV. Consequently, it is possible to effectively control *T. leucotreta* to levels acceptable for export of produce to markets that regulate this pest as a quarantine organism, using a combination of SIT and virus.

POSTER SESSION **V-P9-STU**

Whole genomic sequencing and analysis of Rhagastis binoculata nucleopolyhedrovirus (NPV) in Taiwan

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The diseased *Rhagastis binoculata* larvae, showed a typical symptom of nucleopolyhedrosis, were collected in Taiwan. Based on the light microscope observation and PCR check, the nucleopolyhedrovirus (NPV) infection was confirmed. A phylogenetic analysis based on *lef-8* and *lef-9* indicated that this NPV is a Group II NPV that is closely related to Clanis bilineata NPV (ClbiNPV), besides, the Kimura-2-parameter (K-2-P) value is c.a. 0.05 between this NPV and ClbiNPV, therefore, this NPV was provisionally named "RhbiNPV". Through the NGS approach, the whole genome of RhbiNPV was sequenced and the genomic size of RhbiNPV is 128,899 bp with a 37.16% GC content. There are total 135 putative ORFs. The average ORF identities among RhbiNPV to ClbiNPV, Lymantria dispar nucleopolyhedrovirus (LdMNPV), Autographa californica nucleopolyhedrovirus (AcMNPV) and Cydia pomonella granulovirus (CpGV) are 89.5 %, 39.3 %, 32.8 % and 26.4 %, respectively. Though the RhbiNPV shares similar genomic features with ClbiNPV, there is a genomic fragment showed inverse orientation between Orf-36 to Orf-104 of RhbiNPV to those of ClbiNPV. Based on these results, the RhbiNPV is a new NPV species found in the larvae of Sphingidae.

POSTER SESSION **V-P10-STU**

Improving recombination efficiency in the baculovirus expression system for foreign gene expression

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The baculovirus expression system is extensively used for producing recombinant proteins in insect cells for purposes such as vaccine production. The current technology involves cloning a viral antigen gene into a transfer plasmid prior to introducing the virus vector (*flash*BAC) and transfer plasmid into insect cells, where recombination events transfer the gene into the viral vector genome. When producing vaccines, it is desirable to optimise yield, solubility and ease of purification. With modern technology, each variant has to be cloned into a transfer plasmid. For multiple targets, this process becomes labour-intensive and time-consuming. It would be better if a synthetic fragment could be used to produce recombinant baculoviruses without the sub-cloning step.
Synthetic, linear DNA fragments encoding *eGFP* or *lac*Z were designed with flanking regions homologous to the intended insertion site within *flash*BAC. These flanking regions varied in size from 50-300bp to determine the minimum sequence required for recombination in insect cells. Reducing the flanking sequences to a minimum length allows for a more cost-effective process. The fragments were then combined with *flash*BAC GOLD DNA and used to co-transfect insect cells. The virus DNA was also linearised prior to use to see if this might enhance the production of recombinant viruses. Progeny virus output was assessed by qPCR and plaque-assay at different times post-transfection. Recombinant protein production was also assessed.
Although decreasing the length of recombination arms correlated with lower yields of P0 virus, even the shortest sequences resulted in sufficient infectious particles to derive sufficient P1 virus stock for subsequent protein expression studies. Linearising the virus DNA appeared to have a positive effect on recombination efficiency. These results may pave the way for a 'plug and play' system to increase the agility, speed, and reduce the cost of the initial stages of the *flash*BAC technology.

POSTER SESSION **V-P11-STU**

Transmission of nudivirus among *Oryctes rhinoceros* at the different developmental stages

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Oryctes rhinoceros is a tropical insect native to South-East Asia and South Asia. The eggs were laid in rotting palm trees or compost and develop to adults. In the Pacific Islands, palm trees are a significant resource for people's livelihoods, and these feeding damages by adult *O.rhinoceros* are subjects that need to be solved. Biological control using of Oryctes rhinoceros nudivirus (OrNV), the natural enemy of *O. rhinoceros* discovered in 1963, has been effective for reducing damages of coconut trees where OrNV has been released. However, the detailed mechanisms of this system are not well understood. In this study, we used *O. rhinoceros* from those stages, larvae and adults, transmission of OrNV were quantified from "donar" as infected individuals to "recipient" as non-infected individual to speculate the feasible route of transmission of OrNV.
In this study, donor larvae and adult worms were inoculated with virus, respectively, and the virus levels of recipients were measured by qPCR by rearing them one by one in the same cage with healthy worms (recipients) and comparing which pathway most affected virus dispersal in OrNV.
When we compared the virus production per an adult or a larvae against the adults and larval virus inoculum, it's was shown that that of larvae are higher than that of adults and larvae. However, the combination of adult donor and adult recipient had the highest amount of virus in the recipient. Under these conditions, transmission between adult worms was most likely to occur than between other stages. The result indicated that larvae may be more susceptible to the virus than adults, because the number of copies of virus possessed by larvae at "donor" was higher than that of adults despite adults have a higher amount of viral inoculum than larvae. Also, the amount of virus possessed by "recipient" of larvae was lower than that of adults and it's shown that the transmission between adults was highest and that between different growth stages was unlikely to occur. The results of these transmission routes can contribute to understanding the success or failure of biological control with OrNV.

POSTER SESSION **V-P12-STU**

Quality control of baculovirus propagation based on single nucleotide variants (SNVs)

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Molecular research but also the registration of baculoviruses for insect pest management depends on the use of well characterized baculovirus strains. Although there is no general concept what a virus strain is, a basic requirement is the stable inheritance of strain-specific trait(s) to be used for identification. In the past, such traits were

immunological characters or molecular properties, such as DNA restriction patterns or genome sequences. Ideally, the successful propagation of a given baculovirus strain results in the genetic identity of the virus inoculum and the virus progeny. As baculovirus strains are propagated either *in vitro* (cell culture) or *in vivo* (host larvae), determining the molecular composition is crucial to ensure strain identity. On the example of the Cydia pomonella granulovirus (strain CpGV-S), we employed next-generation sequencing to monitor the identity of the inoculum virus and the virus progeny propagated in host larvae. Single nucleotide variants (SNVs) were used to determine the genotype composition CpGV-S propagations. Viral DNA was isolated, and whole genomes were sequenced using Illumina technology. Raw data underwent quality control and alignment against a common reference. SNV sites were identified, and the relative frequency of the reference nucleotide was plotted for genotype integrity analysis. It was found that infection studies using CpGV-S frequently resulted in different mixed infections with another genotype which might have been activated from a covert infection in the host larvae. The use of SNVs in strain characterization is of unprecedented accuracy. Our findings urge for a thorough quality control when baculovirus strains are propagated because biological parameters, e.g. virulence, may be affected. Our results highlight the importance of monitoring genotypic composition when propagating baculoviruses in host larvae.

POSTER SESSION **V-P13**

Development of a multiplex PCR assay for the detection of nudiviruses in *Drosophila suzukii* using artificial positive controls

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The Spotted Wing Drosophila, *Drosophila suzukii*, is a pest of soft-skinned fruits, posing a significant challenge for fruit and wine growers worldwide. It causes substantial economic losses each year. Current pest management options, mainly based on chemical insecticides, are inefficient and not sustainable, thus more biocontrol methods are needed. Nudiviruses may be candidates for the biocontrol of *D. suzukii* due to their entomopathogenic properties. An example of the use of nudiviruses is the control of the coconut rhinoceros beetle (*Oryctes rhinoceros*) in oil palm plantations. Two distinct nudiviruses, Drosophila innubila nudivirus (DiNV) and Kallithea virus (KV), have been identified from *Drosophila innubila* and *Drosophila melanogaster*, respectively. Infection with DiNV results in reduced fecundity and a significantly decreased lifespan, while infection with KV impairs fly motility and delays egg laying in females. Additional three genome sequences of Drosophila nudiviruses were obtained from sequencing data only: Esparto nudivirus (EV), Tomelloso nudivirus (TV) and Mauternbach nudivirus (MV). In this study, we aim to screen for the presence of nudiviruses in samples of wild *D. suzukii* using PCR analysis.
For PCR reactions, a positive control is required to ensure accuracy. Hence, we designed and artificially synthesized species-specific positive controls based on gene *p74* of KV, DiNV, EV, TV and MV. The partial *p74* sequences were cloned into plasmids and further used as template DNA in PCR reactions instead of actual virus DNA. Moreover, alignments of the five nudivirus *p74* genes were used to identify conserved gene regions suitable for PCR oligonucleotide locations to design primer pairs binding specifically to the *p74* gene regions of the five nudiviruses. The artificially produced DNA templates serve as positive controls for a multiplex PCR analysis to screen samples of *D. suzukii* for nudiviruses in a high-throughput analysis.

POSTER SESSION **V-P14**

Covert infections caused by cypovirus in Noctuidae and Erebidae soybean pests

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Covert infections have significant practical implications as they affect insect rearing for research purposes, insecticide control processes, and the biomass production of edible insects. In Brazil, insect rearing experienced an exponential growth in the last decade to produce biological agents and standardize both bio and conventional insecticides. Given the prevalence of latent infections, we investigated the use of stressor agents to assess the occurrence of viral covert infections in Noctuoidea hosts. Among the stressor agents tested, including copper sulfate, sodium selenite, iron sulfate and *Bacillus thuringiensis*, those most effective in promoting Cypovirus infection were identified. However, sodium selenite exhibited toxicity, resulting in high mortality rates, followed by iron sulfate. The prevalence of CPV infection was highest in *Anticarsia gemmatalis*, followed by *Rachiplusia nu* and *Chrysodeixis includens*, when considering the total number of insects with the diseases. No CPV was detected in *Spodoptera frugiperda*.

POSTER SESSION **V-P15**

Identification of mycoviruses through the transcriptomic data of entomopathogenic fungi, *Beauveria bassiana* NCHU-271 and *Metarhizium pinghaense* NCHU-125

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Mycoviruses have been described as widespread across fungal species, including entomopathogenic fungi (EPF). In this study, mycoviruses belonging to the Narnaviridae and Partitiviridae families were found in the transcriptomic data of *Beauveria bassiana* NCHU-271 (Bb-NCHU-271) and *Metarhizium pinghaense* NCHU-125 (Mp-NCHU-125). In Bb-NCHU-271, it was found to be infected by a narnavirus (Bb-271-NV), which has one RNA genome of 1,720 bp in size and has putative polypeptide encoding the RdRP. Besides, Bb-271-NV has three subviral RNAs (SRs), and the SRs termini showed high identities to those of Bb-271-NV. Interestingly, Bb-NCHU-271 was also infected by a partitivirus (Bb-271-PV), which has two RNA genomic segments (dsRNA1= 1,790 bp and dsRNA= 1598 bp). The dsRNA1 has putative ORF1 encodes the RdRP. The dsRNA2 has putative ORF2 encodes the coat protein (CP). In Mp-NCHU-125, a polymycovirus (Mp-125-PmV) was found. The RNA genome consists of four dsRNAs with the sizes of 2400bp (ORF1, RdRP), 2332bp (ORF2), 1920bp (ORF3, Methyltransferase) and 1351bp (ORF4, PAS-rich protein). The RdRP of three mycoviruses phylogenetically clustered with Beauveria bassiana narnavirus, Beauveria bassiana partitivirus 1 and Metarhizium anisopliae polymycovirus 1. The presenting of mycoviruses were validated by RT-PCR. The single spore isolation method will be applied to evaluate its impact on EPF.

POSTER SESSION **V-P16**

The importance of ORF amv*133* on Amsacta moorei entomopoxvirus replication

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Amsacta moorei entomopoxvirus (AMEV) is an insect entomopoxvirus that infects Lepidopteran (moths) and Orthopteran (grasshoppers) insects and is a potential microbial control agent. *amv133* is an AMEV gene that encodes an active esterase enzyme with protease activity. Viral genes encoding lipolytic proteins and proteases play a role in various functions, including producing DNA replication metabolites, rescue from endosomes, membrane fusion, transcriptional regulation, and insecticidal activity. We explored the significance of *amv133* on AMEV growth, viral DNA replication and expression of immunity-related genes. For this purpose, an *amv133* knockout virus was constructed using homologous recombination, and the virus titre and viral DNA accumulation at 0, 3, 6, 12, 24, 48 and 72 hours post-infection were compared to endpoint dilution assay and quantitative PCR, respectively. Surprisingly, the deletion of *amv133* resulted in a 3.6- and 1.8-fold increase in infectious budded virus titre and viral DNA production in tissue culture, respectively. Changes in transcript levels of some host immunity-related genes, compared to uninfected cells, were also monitored following infection with wild-type or *amv133* knockout virus-infected Ld652 cells to determine which transcriptional changes could be attributed to the presence of the AMV133 protein.

POSTER SESSION **V-P17**

Developing potential intervention strategies for SFTSV group bandaviruses based on their phylogeny

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Severe fever with thrombocytopenia syndrome virus (SFTSV) is a novel tick-borne virus that causes SFTS disease with a mortality rate of up to 30% found in the central and eastern China, and later in South Korea and Japan. SFTSV phylogeny is associated with geographical regions, with Chinese strains primarily belonging to the C (C1-C5) genotype, and Korean and Japanese strains belonging to the J (J1-J3) genotype. Heartland virus (HRTV), reported in the United States, and Guertu virus (GTV), isolated from ticks in Xinjiang, China, are phylogenetically close and share high sequence identities with SFTSV, and all belong to a group of the genus Bandavirus in the family Phenuiviridae of the order Bunyavirales. Here, bioinformatic analyses identified the hotspot mutations among SFTSV genotypes and suggested the increasing genetic diversity by presenting new genotypes. This may challenge the development of detection techniques, specific drugs, and vaccines against SFTSV. Monoclonal antibodies (mAbs) targeting nucleoprotein (NP) and/or glycoprotein (GP) of SFTSV, GTV, or HRTV were then generated. Four mAbs targeting the GTV NP showed cross-reactivity to SFTSV, demonstrating the potential to be developed as new detection methods for both viruses. Three mAbs derived from SFTSV GP, three others from GTV GP, and two from HRTV GP showed different efficiencies to recognize SFTSV, HRTV, and GTV respectively. Of these mAbs, one derived from SFTSV GP possessed neutralizing activity against both SFTSV and GTV and conferred protection against mortality in mice, suggesting that this mAb is a potential candidate for development as a drug for disease therapy. Our results promote the understanding of the evolutionary relationships of SFTSV group bandaviruses. The different abilities of mAbs to cross-react between SFTSV, GTV, and HRTV, in line with the genetic diversities between them, would further benefit designing and developing strategies for disease intervention and therapy.

POSTER SESSION **V-P18-STU**

Expression of respiratory syncytial virus fusion protein using an insect virus surface-display system

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Respiratory syncytial virus (RSV) is a deadly respiratory disease that primarily affects infants and the eldery, and the only treatment is the passive administration of antibodies. Fusion proteins of RSV are considered excellent vaccine targets due to their high structural stability and ability to induce high levels of neutralizing antibodies. Baculovirus surface-display system is a valuable protein engineering technology that allows the target proteins to be anchored on the surface of host cells and viruses in trimeric form. However, this system has so far only used basic promoters with low target protein production, and there has been little research on increasing the efficiency of surface-display through promoter enhancement. Therefore, in this study, we evaluated the surface-display efficiency of various promoters to select the optimal promoter that can increase the production of surface-display proteins, and used the selected promoter to perform surface display of RSV-F protein, an antigenic protein that has not been previously expressed through baculovirus surface-display.

POSTER SESSION **V-P19**

Viromes of tick tissues revealed different capabilities to vector and transmit viruses in nine tick species.

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Ticks are important vectors for carrying and transmitting viruses. They acquire viruses from hosts into their midgut by sucking blood. If a virus passes through the intestinal wall, it may reach and proliferate in the salivary gland and/or ovary. This virus can then be transmitted horizontally to other hosts via bloodsucking or vertically to the next generation via laying eggs. However, the role of tick tissues in vectoring and transmitting viruses remains unclear. This study examined the viromes of the midgut, salivary gland, and ovary of nine tick species belonging to six genera. A total of 161 genomic sequences of viruses from more than 16 virus families were identified. Haemaphyalis had the highest viral abundance, while Rhipicephalus exhibited the greatest viral diversity. The ovary had a higher viral abundance than the midgut and salivary gland, suggesting a greater role in maintaining virus community. The virus abudance in the ovary of Rhipicephalus (Boophilus) and Rhipicephalus was significantly higher than in the midgut and salivary gland. Although some viruses are commonly found across all three tissues of varied abundance, indicating a broad tissue tropism of these viruses in ticks, there are also viruses that are only detected in one tissue, suggesting tissue specificity in maintaining viruses. Moreover, the abudance of the viruses in ovary of Rhipicephalus, Ixodes, Demacentor, and Phipicephalus (Boophilus) was significantly positively correlated with that in the salivary glands, indicating a common mechanism of virus transmission within these ticks. The results suggested the potential roles of the ovary, midgut, and salivary gland in the horizontal or vertical transmission of viruses and the spread of viruses within tick bodies. The findings improve our understanding of tick tissues in the transmission of viruses and could aid

further research into the mechanisms of virus spread among different tick species.

POSTER SESSION **V-P20-STU**

Estimation of regions responsible for the lethal activity of parasitoid killing factors encoded in entomopoxvirus by point mutation

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Parasitoid Killing Factor (PKF) is encoded in the genome of insect viruses and lepidopteran insects. When infection of Mythimna separata entomopoxvirus (MySEV) and parasitization of a hymenopteran parasitoid, *Cotesia kariyai*, co-occurred in the same host, *Mythimna separata* larva, the parasitoid larvae are killed in the host larvae. PKF protein was also identified from the hemolymph of MySEV-infected *M. separata* larvae as a toxic protein against parasitoid larvae. In previous studies, PKF has been shown to induce apoptosis to cells derived from *C. kariyai* (CK1). In addition, PKF protein is presumed to be cleaved near the center and divided into a cysteine-rich N-terminal region and a C-terminal region containing a putative endonuclease domain. However, the function of N- and C-terminal regions in PKF toxicity is unknown. In this study, recombinant baculoviruses inserted with PKF genes were constructed and inoculated *M. separata* larvae. Soluble PKF was expressed in the larvae and was toxic to CK1 cells. To elucidate whether the endonuclease domain is involved in the toxicity, we also expressed PKF with mutations in several residues, including the putative catalytic residues, and the effect of the mutations on toxicity was examined. Consequently, mutation of the putative catalytic residues resulted in loss of PKF toxicity, suggesting that the endonuclease domain is responsible for the toxicity of PKF against CK1.

POSTER SESSION **V-P21-STU**

Development of a DWV-specific immunohistochemistry protocol to analyze tissue and cell tropism of DWV

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Infections with deformed wing virus (DWV) are one of the major threats to honey bees, as clinically relevant DWV-infections in conjunction with *Varroa destructor* infestation correlate significantly with honey bee colony losses. Overt DWV infections depend on the transmission of the virus to bee pupae by *V. destructor* and are characterized by pupal death or adult bees emerging with deformed wings and a shortened abdomen. To better understand the pathogenesis of DWV infections, we aimed at identifying tissues and cells which can be infected by DWV und permit replication of the virus. We previously established fluorescence *in situ* hybridization (FISH) analysis for the detection of viral RNA infected cells and here aimed at developing a DWV-specific, antibody-based immunohistochemistry protocol for the detection of virus particles in infected tissues. To this end, firstly, we recombinantly expressed and then purified the capsid protein recDWV-VP2 for the commercial (ASKA Biotech) production of monoclonal antibodies. One of the antibodies obtained showed high specificity for the recDWV-VP2 antigen in dot blots and Western blots and was able to distinguish between bees that had tested DWV- positive and DWV- negative in RT-PCR assays. We next tested this antibody in immunohistochemistry using sections of brain, thorax and intestine of crippled (DWV-positive in PCR) and healthy (symptomless and DWV-negative in PCR) bees. DWV was detected in the epithelial cells of the intestine and in the cells of various regions of the brain of crippled bees. In thorax sections, signals were detected only in the connective tissue surrounding the muscle cells, the perimysium and endomysium, but not inside the muscle cells. These results confirm and extend previous findings on midgut- and neurotropism of DWV by showing clear cell tropism: DWV can infect epithelial and neuronal cells, but not muscle cells.

POSTER SESSION **V-P22**

Use of Insect Gut Binding Peptides to Identify Pathogen Binding Domains and Receptors

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The majority of insect pathogens enter the host via the gut. However, our knowledge of the molecular interactions between pathogens and the surface of the insect gut epithelium is remarkably limited. Indeed, only five pathogen receptors have been definitively identified in the insect gut. Receptors that mediate the entry of key viruses of agricultural and human health importance, including the many viruses that are vectored by mosquitoes, are unknown. A powerful approach to overcome these knowledge gaps is the use of insect gut binding peptides to identify 1) binding domains on pathogen surface proteins, and 2) receptors on the surface of the insect gut epithelium that mediate pathogen entry. Here we describe use of this approach to identify the honey bee gut receptors of *Israeli acute paralysis virus* (*Dicistroviridae*). We also highlight the availability of gut binding peptide datasets for multiple insect species for others to explore the molecular interactions of pathogens of choice with the proteins on the surface of the host insect gut.

POSTER SESSION **V-P23-STU**

Efficient production of hand, foot, and mouth disease virus-like particles (HFMD-VLPs) in insect cells

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Hand, foot, and mouth disease (HFMD), characterized by rashes on the hands and feet and ulcers in the mouth, is highly contagious and has no specific treatment. It mostly affects infants and children under the age of five, who are immunocompromised, so there is a need to develop a safe vaccine. Virus-like particles (VLPs) are particles with a virus-like structure that lack genetic material, making them unable to replicate and infect. This is why VLPs are considered to have a high level of biological safety and have excellent value as vaccines. In this study, baculovirus expression system (BES), which has been widely used in vaccine and pharmaceutical production due to its high post-translational modification efficiency, was used to produce VLPs for Coxsackievirus types, which are recently reported to be the main cause of HFMD. For efficient production of VLPs, it is necessary to regulate the expression quantity and timing of the 3CD protein, which is essential for VLPs production by cleaving structural proteins, but has been reported to be cytotoxic. Therefore, the expression of 3CD protein was controlled by various promoters, and the optimal expression form of HFMD-VLPs was determined by comparing the resulting cleavage efficiency.

Development of a Bioinformatic Pipeline for Defining Orthology Groups in *Baculoviridae* Protein Genes.

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Baculoviridae is a family of insect viruses with circular double-stranded DNA genomes that have significant biotechnological applications. They are classified into four genera (*Alpha*-, *Beta*-, *Gamma*-, and *Deltabaculovirus*) and carry up to 183 gene proteins. While core proteins, derived from 38 shared genes among all baculoviruses, have been extensively studied, other shared protein sets exist among genera, alongside unique genes. Novel bioinformatic analyses could illuminate baculovirus proteomics, potentially aiding in the biotechnological applications derived from these entities. Therefore, this study proposes a bioinformatic pipeline for characterizing baculoviral proteomes and postulating ortholog groups, employing different bioinformatic tools. Our database was based on 297 genomes recovered from GenBank. Two ortholog determination tools, ProteinOrtho and OrthoFinder, were initially employed, but complete "core protein" set detection was deficient. Further analysis utilized various tools for homology sequence search (Diamond, BlastP, Psi-Blast, tBlastN) and hidden Markov models (HMMs, via HMMER). The final algorithm involves initial ortholog group determination with ProteinOrtho, followed by iterative searches with HMM-generated multiple alignments on a selected genome database, with additional searches using tBlastN for unannotated proteins. This approach successfully detected all 38 "core proteins" across all genera. Furthermore, the algorithm postulated a series of proteins (Major-OB-Protein, Lef11, Ac106/107, and Ac131) in which only the deltabaculovirus homologue was absent. Subsequent exhaustive studies on non-assigned CuniNPV protein-genes allow us to detect four ORFs as putative remote orthologs, and postulate that the Major-OB-Protein may be considered as the 39th core gene. These advances could redefine the number of baculovirus ancestral protein genes, which may be useful to better understand the infectious cycle of these entomopathogens.

AUTHOR INDEX

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