LE STUDIUM

CONFERENCES

VIRTUAL MEETING | 2021

28th June - 2nd July 2021

2021 International Congress on Invertebrate Pathology and Microbial Control & 53rd Annual Meeting of the Society for Invertebrate Pathology

LOCATION

VIRTUAL MEETING

CONVENORS

Dr Cristina Del Rincon Castro

LE STUDIUM / MARIE SKŁODOWSKA-CURIE RESEARCH FELLOW

FROM University of Guanajuato - Mexico

IN RESIDENCE AT Insect Biology Research Institute (IRBI), University of Tours / CNRS - FR

Dr Elisabeth Herniou

Insect Biology Research Institute (IRBI), University of Tours / CNRS - FR

LE STUDIUM Loire Valley Institute for Advanced Studies

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ABSTRACTS

2021 International Congress on Invertebrate Pathology and Microbial Control & 53rd Annual Meeting of the Society for Invertebrate Pathology

CONVENORS

Dr Cristina Del Rincón Castro
LE STUDIUM / MARIE SKŁODOWSKA-CURIE RESEARCH FELLOW
FROM: University of Guanajuato - México
IN RESIDENCE AT: Insect Biology Research Institute (IRBI), University of Tours / CNRS - FR

Dr Elisabeth Herniou
Insect Biology Research Institute (IRBI), University of Tours / CNRS - FR

ORGANIZING COMMITTEE

Sophie Gabillet, General Secretary

Dr Aurélien Montagu, Scientific Relations Manager

Maurine Villiers, Communication & Events Manager

LE STUDIUM Loire Valley Institute for Advanced Studies • Région Centre-Val de Loire • FR

EDITO

Created in 1996 on the CNRS campus in Orleans La Source, LE STUDIUM has evolved to become the multidisciplinary Loire Valley Institute for Advanced Studies (IAS), operating in the Centre-Val de Loire region of France. LE STUDIUM has its headquarters in the city centre of Orleans in a newly renovated 17th century building. The amazing facilities are shared with the University of Orleans. In 2014 new developments and programmes linked to the smart specialisation of the Centre-Val de Loire region came to strengthen existing IAS collaborative relationships with the local and the international community of researchers, developers and innovators.

LE STUDIUM IAS offers to internationally competitive senior research scientists the opportunity to discover and work in one of the IAS's affiliate laboratories from the University of Tours, the University of Orleans, National Institute of Applied Sciences (INSA) Centre Val de Loire and ESAD Orléans, as well as of nationally accredited research institutions located in the region Centre-Val de Loire (BRGM, CEA, CNRS, INSERM, INRAE). Our goal is to develop and nurture trans-disciplinary approaches as innovative tools for addressing some of the key scientific, socio-economic and cultural questions of the 21st century. We also encourage researchers' interactions with industry via the IAS's links with Poles of Competitiveness, Clusters, Technopoles, and Chambers of Commerce etc.

LE STUDIUM has attracted two hundred and thirty experienced researchers coming from 47 countries for long-term residencies. In addition to their contribution in their host laboratories, researchers participate in the scientific life of the IAS through attendance at monthly interdisciplinary meetings called LE STUDIUM THURSDAYS. Their presentations and debates enrich the regional scientific community at large (researchers of public and private laboratories, PhD students, research stakeholders' representatives, etc...)

For the period 2015-2021, LE STUDIUM operates with an additional award from the European Commission in the framework of the Marie Skłodowska-Curie Actions (MSCA)-COFUND programme for the mobility of researchers. Since 2013, LE STUDIUM is also an official partner of the Ambition Research and Development 2020 programmes initiated by the Centre-Val de Loire Regional Council to support the smart specialisation strategy (S3) around 5 main axes: biopharmaceuticals, renewable energies, cosmetics, environmental metrology and natural and cultural heritage. New programmes are currently designed to include all major societal challenges.Researchers are also invited and supported by the IAS to organise, during their residency and in collaboration with their host laboratory, a two-day LE STUDIUM CONFERENCE. It provides them with the opportunity to invite internationally renowned researchers to a cross-disciplinary conference, on a topical issue, to examine progress, discuss future studies and strategies to stimulate advances and practical applications in the chosen field. The invited participants are expected to attend for the duration of the conference and contribute to the intellectual exchange. Past experience has shown that these conditions facilitate the development or extension of existing collaborations and enable the creation of productive new research networks.

The present LE STUDIUM CONFERENCE named is "*2021 International Congress on Invertebrate Pathology and Microbial Control & 53rd Annual Meeting of the Society for Invertebrate Pathology*" the 111th in a series started at the end of 2010 listed at the end of this booklet.

We thank you for your participation and wish you an interesting and intellectually stimulating conference. Also, we hope that scientific exchanges and interactions taking place during this conference will bring opportunities to start a productive professional relationship with presenting research laboratories and LE STUDIUM Loire Valley Institute for Advanced Studies.

Yves-Michel GINOT

Chairman
LE STUDIUM

INTRODUCTION

Since 1967, the Society for Invertebrate Pathology (SIP) has brought together members from diverse scientific backgrounds under the unified discipline of invertebrate pathology. The SIP has 7 Divisions: Bacteria, Diseases of Beneficial Invertebrates (DBI), Fungus, Microbial Control, Microsporidia, Nematode and Virus, which promote scientific knowledge of pathology of invertebrate animals, including pest species and species of commercial interest.

Despite the current pandemics, the Society for Invertebrate Pathology has decided to hold its annual meeting but this should take place on line. It is therefore our pleasure to invite you to attend the **2021 International Congress on Invertebrate Pathology and Microbial Control & SIP2021 - the 53rd Annual Meeting of the Society for Invertebrate Pathology** to be held **virtually from 28th of June to 2nd of July 2021 in Tours, Loire Valley, France** with the participation of Le Studium, IRBI, CNRS, University of Tours and the University of Guanajuato (Mexico).

As always, the meeting will offer an exciting **scientific program** exploring the latest fundamental and applied findings in invertebrate pathology, including microbial control, diseases of beneficial invertebrates, and advances in fundamental research on host-pathogen interactions. Among the scientific highlights, the society's divisions have put together stimulating symposia and the plenary symposium will be on '**Current challenges for the microbial control of Spodoptera frugiperda**'. All symposia will be held live on the zoom platform and pre-recorded contributed talks and posters posted for viewing prior to live chat sessions.

This conference is organised in partnership with **EntomoCentre**, a Research Thematic Network, focused on Entomology and funded by the Centre-Val de Loire Region. The network aims to promote studies in entomology in relation to global changes and innovative technologies.

PROGRAMME AT A GLANCE

Contributed presentations on VOD and posters will be made available to registered participants for early view from the conference website, as soon as they are posted

MONDAY 28TH JUNE

00:00-23:59 Contributed papers on VOD

00:00-23:59 Posters papers to view at leisure

13:00-13:30 **Opening Ceremony**

13:30-15:00 **Plenary Symposium** *Current challenges for the microbial control of Spodoptera frugiperda*

15:00-15:15 Break

15:15-16:45 **Plenary Symposium** *Current challenges for the microbial control of Spodoptera frugiperda*

16:45-17:00 Break

17:00-19:00 **Diseases of Beneficial Invertebrates Divisional Symposium**
Pathological advances in carcinology

TUESDAY 29TH JUNE

00:00-23:59 Contributed papers on VOD

00:00-23:59 Posters papers to view at leisure

13:15-13:45 **Chat sessions**
DBI: Diseases of Beneficial Invertebrates
MCO: Microbial Control with Virus
NEM: Nematodes as model in applied biology and soil ecology

13:45-14:00 Break

14:00-16:00 **Microbial Control Division Symposium** *Promising microbial control options for fall armyworm, a global perspective*

16:00-16:15 Break

16:15-16:45 Chat sessions

FUN: Entomopathogenic fungi diversity 1
VIR: Advances in Insect molecular virology

16:45-17:00 Break

17:00-19:00 Virus Division Symposium *Place of baculoviruses in the fight against Covid-19*

19:00-19:15 Break

19:15-19:45 Chat sessions

BAC: Pathogen physiology
FUN: Entomopathogenic fungi diversity 2
VIR: Advances in Insect molecular virology

WEDNESDAY 30TH JUNE

00:00-23:59 Contributed papers on VOD

00:00-23:59 Posters papers to view at leisure

13:15-13:45 Chat sessions

BAC: Pesticidal Protein Mode of Action
FUN: Physiological Interactions
VIR: Host-pathogen interactions

13:45-14:00 Break

14:00-16:00 Fungi Division Symposium *New Advances in the World of the Entomophthorales*

16:00-16:15 Break

16:15-16:45 Chat sessions

FUN: Applied Aspects 1
MIC: Insect microsporidia: host pathology and disease control
VIR: Endogenous viruses

16:45-17:00 Break

17:00-19:00 Microsporidia Division Symposium *Microsporidia of invertebrate hosts in aquatic and terrestrial habitats*

19:00-19:15 Break

19:15-19:45 Chat sessions

DBI: Insect as Food and Feed
MCO: Microbial Control with Proteins
NEM: Nematodes as model in applied biology and soil ecology

THURSDAY 1ST JULY

00:00-23:59 Contributed papers on VOD

00:00-23:59 Posters papers to view at leisure

13:15-13:45 Chat sessions

MCO: Microbial control interactions
MIC: Microsporidia biodiversity and physiology
VIR: Virus detection and identification

13:45-14:00 Break

14:00-16:00 Diseases of Beneficial Invertebrates & Virus Cross-Division Symposium *Viruses of Pollinators*

16:00-16:15 Break

16:15-16:45 Chat sessions

BAC: Receptors and resistance
FUN: Applied aspects 2
NEM: Advances in formulation, application and control of pests
VIR: Viral bioinsecticide

16:45-17:00 Break

17:00-19:00 Bacteria Division Symposium *Analysis of Vip3A and Cry protein mechanism of action*

19:00-19:15 Break

19:15-19:45 Chat sessions

BAC: Strains and proteins
MCO: Microbial control with fungi

FRIDAY 2ND JULY

00:00-23:59 Contributed papers on VOD

00:00-23:59 Posters papers to view at leisure

13:30-15:30 **Nematode Symposium** *Entomopathogenic nematodes or scavengers: Revisiting the emerging new nematodes classified as EPN*

15:30-15:45 Break

15:45-17:15 **SIP Awardee Symposium** *Martignoni Award 2021*
Early Career Award 2020 & 2021

17:15-17:30 Break

17:30-18:30 **SIP Business Meeting** *Announcement of Student Prizes*

18:30 **Meeting Closure**

Abbreviations for the different divisions:
BAC: Bacteria; DBI: Diseases of Beneficial Invertebrates; FUN: Fungi; MCO: Microbial Control; MIC: Microsporidia; NEM: Nematode; VIR: Virus

SIP PRESIDENT

Dr Christina Nielsen-LeRoux

INRAE, University Paris Saclay

4 Avenue Jean Jaures, Micalis
78350 Jouy en Josas - FR

Email:Christina.nielsen.Leroux@inrae.fr
Phone: 33(+) 1 34 65 2101

C. Nielsen-LeRoux is senior scientist at INRAE (National Research Institute for Agriculture, Food and Environment) at the Micalis institute. Her actual aera of interest deals with entompathogenic bacteria: Bacillus thuringiensis and the closely related opportunistic human and insect pathogen B. cereus. Identification of effectors responsible for insect adaptation and virulence, (cry toxin's interaction with intestinal barriers (peritrophic matrix) and bacterial iron homeostasis factors expressed during the host (model Galleria mellonella) infection are among the main topics. More recently she became involved in research related to the health of Insects for feed and food and the microbial risk issues of such insects in the food chain. She is the actual (2020-2022) president of the SIP.

What SIP stands for:.

The Society for Invertebrate Pathology (SIP) was founded in 1967 in USA as an interdisciplinary scientific society that would draw together members from diverse scientific backgrounds under the unified discipline of invertebrate pathology. The SIP is highly international, members are from about 30 countries, while the majority are still from USA, Canada and Europe.

The objectives as defined in the Society's constitution are:

- Promotion of scientific knowledge of pathology of invertebrate animals and of related subjects through discussions, reports and publications.
- Stimulation of scientific investigations and their applications
- Planning, organization and administration of projects for the advancement of scientific knowledge in invertebrate pathology
- Improvement of education and of professional qualifications in invertebrate pathology
- Promotion of international cooperation in achieving the above objectives.

Now 53 years later the SIP is still the place to be to meet people and science on high level in the field of «invertebrate pathogens» (virus, fungi, nematodes, microsporidia, bacteria). We have a websitehttp://www.sipweb.org/about/about.html) with links to specific topics and person dealings with each pathogenic group. The community meets annually (as this conference) in different parts of the world to share new results in the domains of microbial control of invertebrate pest, in the control of diseases of beneficial invertebrate (bee's, Insect for feed and food, seafood etc.).The mode of action and the mechanisms underlying the pathogenesis and many other fundamental aspect of host pathogen interactions are typical aspects highlighted for each kind of the pathogens or domains (we have 7 divisions). During the SIP meeting's the atmosphere is really nice, like a family, special sessions are organized for students. We have competitions, excursions and often special hot topic workshops are organized as well.

We hope that this virtual meeting will be the start for new members to join the SIP for several upcoming years, as for sure it will be an appetizer for the normal SIP live meeting. The one in 2022 is planned for South-Africa. Welcome to SIP, if you want to know more see the website and contact :

E-mail: sip@sipweb.org Verona, WI 53593 USA
Society for Invertebrate Pathology Christina Nielsen-LeRoux
PO Box 930082 SIP President (2020-2022)

SIP OFFICERS

Division of Bacteria

Chair ..... Omaththage Perera
Past Chair ..... Marianne Pusztai-Carey
Chair Elect ..... Colin Berry
Secretary/Treasurer ..... Neil Crickmore
Past Secretary/Treasurer ..... Shuyuan Guo
Members-at-Large ..... Luca Ruiu
Student Representative ..... To be announced

Division of Diseases of Beneficial Invertebrates

Chair ..... Mark Freeman
Past Chair ..... Helen Hesketh
Chair Elect ..... Grant Stentiford
Secretary/Treasurer ..... Helen Hesketh
Past Secretary/Treasurer ..... Kelly Bateman
Members-at-Large ..... Kelly Bateman, Annette Bruun Jensen
Student Representatives ..... Thomas Gillard, Nicole Atherley

Division of Fungi

Chair ..... Stefan Jaronski
Past Chair ..... Nicolai Meyling
Chair Elect ..... Chengsu Wang
Secretary/Treasurer ..... Louela Castrillo
Past Secretary/Treasurer ..... Ann Hajek
Members-at-Large ..... Jae Su Kim, Senthil Kumar
Student Representatives ..... Kari Zurowski

Division of Microbial Control

Chair ..... Jarrod Leland
Past Chair ..... Dietrich Stephan
Chair Elect ..... Chad Keyser
Secretary/Treasurer ..... Mary Barbercheck
Past Secretary/Treasurer ..... Michael Brownbridge
Members-at-Large ..... Roma Gwynn, Edith Ladurner
Student Representatives ..... Pauline Deschodt, Swati Mishra

Division of Microsporidia

Chair ..... George Kyei-Poku
Past Chair ..... Yuliya Sokolova
Chair Elect ..... Yuri Tokarev
Secretary/Treasurer ..... Andreas Linde
Past Secretary/Treasurer ..... Julie Hopper
Members-at-Large ..... Jie Chen, Sarah Biganski
Student Representatives ..... Artur Trzebny

Division of Nematodes

Chair ..... Raquel Campos-Herrera
Past Chair ..... Glen Stevens
Chair Elect ..... Patricia Stock
Secretary/Treasurer ..... Ivan Hiltpold
Past Secretary/Treasurer ..... Patricia Stock
Members-at-Large ..... Tshima Ramkuwela, Alder Dillam
Student Representatives ..... Diana la Forgia

Division of Viruses

Chair ..... Elisabeth Herniou
Past Chair ..... Madoka Nakai
Chair Elect ..... Rollie Clem
Secretary/Treasurer ..... Adly Abd-Alla
Past Secretary/Treasurer ..... Vera Ros
Members-at-Large ..... Gaelen Burke, Manli Wang
Student Representatives ..... Hiroyuki Hikida, Shili Yang

SIP COMMITTEES

Nominating Committee
Johannes Jehle (Chair), Peter Krell, Jørgen Eilenberg, Leellen Solter, Madoka Nakai

Membership Committee
Peter Krell (Chair), Surendra Dara (ex officio), Edith Ladurner, Tshima Ramakuwela, Gaelen Burke, Sunday Ekesi, Kerstin Jung, Waqas Wakil, Annette Jensen

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David Shapiro-Ilan (Chair), Selcuk Hazir, Albrecht Koppenhöfer, Bryony Bonning, Christina Nielsen-LeRoux (ex officio), Surendra Dara (ex officio), Rose Hu (ex officio), Sreerama Kumar Prakya (ex officio), Cecilia Schmitt (ex officio), Leellen Solter (ex officio)

Meetings Committee
Mark Goettel (Chair), Nina Jenkins, Elisabeth Herniou, Jörg Wennmann

Endowment & Financial Support Committee
Roma Gwynn (Chair), Michael Brownbridge, Mike Dimock, James Harper, Jarrod Leland, Surendra Dara (ex officio)

Founders' Lecture Committee
Neil Crickmore (Chair), Mark Goettel, James Becnel

Awards & Student Contest Committee
Vera Ros (Chair), Andreas Linde, Hyun-Woo Park, Kelly Bateman

History Committee
Stefan Jaronski (Chair), James Harper, Donald Roberts, Harry Kaya, Juerg Huber, Mark Goettel, Just Vlak, Richard Humber

Archivist
Johannes Jehle

Student and Postdoctoral Affairs Committee
Pavan Kumar (Chair, 2017–2019), Daniel Pinos Pastor (Bacteria Division, 2018–2020), Thomas Gillard (Diseases of Beneficial Invertebrates Division, 2018–2020), Rodrigo Lopez Plantey (Fungi Division, 2017–2019), Jiangbin Fan (Microbial Control Division, 2017–2019), Pauline Deschodt (Microbial Control Division, 2018–2020), Paul Airs (Nematode Division, 2017–2019), Bob Boogaard (Virus Division, 2017–2019), Hiroyuki Hikida (Virus Division, 2018–2020), Patricia Stock (Faculty Advisor)

ORGANISATION COMMITTEES

President
Christina Nielsen-LeRoux

Vice President
Patricia Stock

Past-President
Zhihong (Rose) Hu

Secretary
Helen Hesketh

Treasurer
Albrecht M. Koppenhofer

Trustee
Martin Erlandson

Trustee
Kelly Bateman

Trustee
Sassan Asgari

Trustee
Vera Ros

PROGRAMME

MONDAY 28TH JUNE

13:00-13:30 Welcome Address
Dr Elisabeth Herniou and Dr Cristina Del Rincon Castro, Convenors
Ms Sophie Gallibert, Le Studium, France
Dr David Giron, Insect Biology Research Institute, CNRS-Université de Tours and Entomocentre, France
Dr. Mauro Napsuciale Mendívil, University of Guanajuato, Mexico
Dr Christina Nielsen Leroux, President of the SIP

PLENARY SYMPOSIUM: CURRENT CHALLENGES FOR THE MICROBIAL CONTROL OF *SPODOPTERA FRUGIPERDA*(13:30-15:00)

13:30 Dr Buyung Hadi How not to waste a crisis: A pest invasion as an opportunity to scale up biopesticides

14:00 Dr Emmanuelle D'Alençon Know your enemy: Integrative study of plasticity, adaptive evolution and speciation in the Fall armyworm

14:30 Prof Julio Bernal Reclaiming an ancestor's legacy: Fortifying the maize microbiome against fall armyworm herbivory using teosinte microbiota

15:00 - 15h15 Break

PLENARY SYMPOSIUM: CURRENT CHALLENGES FOR THE MICROBIAL CONTROL OF *SPODOPTERA FRUGIPERDA*(15:00- 16:45)

15:15 Dr Trevor Williams Two decades of collaborative research on Spodoptera frugiperda MNPV

15:45 Dr Holly Popham How SfMNPV has moved from a concept to a control method of Spodoptera frugiperda

16:15 Prof Juan-Luis Fuentes Challenges and opportunities for bacterial control of Spodoptera frugiperda

16:45-17:00 Break

DISEASES OF BENEFICIAL INVERTEBRATES DIVISIONAL SYMPOSIUM: PATHOLOGICAL ADVANCES IN CARCINOLOGY(17:00-19:00)

17:00 Dr Andrew Kough Disease slows crawling crabs and alters modeled connectivity between North American Callinectes sapidus populations

17:20 Erin Walters Floridian blue crab (Callinectes sapidus) diseases across freshwater and marine environments

17:40 Elizabeth Duermit-Moreau Diversity and disease of mobile benthic fauna in Florida Bay after harmful cyanobacteria blooms degrade hard-bottom habitat

18:00 Dr Kelly Bateman Emergence of paramoebiasis in edible crabs (*Cancer pagurus*) from UK waters

18:20 Mingli Zhao A widely distributed pathogenic reovirus affecting the Atlantic blue

18:40 Dr Charlotte Davies, Disease connectivity: Investigating disease dynamics in shore crabs, *Carcinus maenas*

TUESDAY 29TH JUNE

CHAT SESSION: DISEASES OF BENEFICIAL INVERTEBRATES (13:15-13:45)

Nicole Atherley, The enemy that lurks: egg-predators of the Caribbean spiny lobster

Dr Kristin Duffiel, Identification and quantification of entomopathogenic viruses in reared crickets

Dr Benjamin Gourbal, Epigenetic and metabolomic changes in hemocytes underlie innate immune memory in the vector snail Biomphalaria glabrata

Pascal Herren, Immune priming in Tenebrio molitor induced by temperature stress and a fungal pathogen

Remi Pichon, A Single Cell RNA sequencing approach to characterize Biomphalaria glabrata hemocyte responses in innate immune memory

Robert Pienaar, First evidence of long-lasting association between viruses and the Black soldier fly, Hermetia illucens

Terri Price, The Consumption and survival rate of *Lilioceris cheni* (Coleoptera:Chrysomelidae) on Air Potato Leaves Exposed to *Cordyceps fumosorosea* (Hyprocreales: Cordycipitaceae)

Dr Marina Querejeta Coma, Drivers and role of bacterial diversity and composition along the developmental stages of the Black Soldier Fly *(Hermetia illucens)*

CHAT SESSION: MICROBIAL CONTROL WITH VIRUS (13:15-13:45)

David Grzywacz, A Novel Formulation for Baculoviruses Protects Biopesticide from

Degradation by Ultraviolet Radiation – Laboratory and Plant Trials with Spodoptera littoralis Nucleopolyhedrovirus Confirms Greatly Extended UV Stability

Ahmed G. Hussain, Developing a sustainable attract and infect strategy for the control of the fall armyworm, *Spodoptera frugiperda*, in Africa

Jie Li, Identification of a PGRP-lb gene in *Spodoptera exigua* with antiviral function against S. exigua multiple nucleopolyhedrovirus

Sean Marshall, Production of Oryctes nudivirus (OrNV) through the DSIR 1179 Heteronychus arator cell line

Dr Sean Moore, Successful selection of a UV-resistant Cryptophlebia leucotreta betabaculovirus for a more persistent biopesticide

Dr Luca Ruiu, LdMNPV baculovirus as a regulator of gypsy moth population dynamics in cork oak forest

CHAT SESSION: NEMATODES AS MODEL IN APPLIED BIOLOGY AND SOIL ECOLOGY (13:15-13:45)

Rubén Blanco-Pérez, Impact of differentiated vineyard management on the activity of entomopathogenic nematodes in La Rioja (Spain)

Maria Cassels, The effects of female pheromone exposure on lethal fighting in *Steinernema carpocapsae* males

Maryam Chelkha, Unraveling the effect of the presence of earthworms or their cutaneous excreta and entomopathogenic nematodes in the soil bacterial community, biocontrol capacity, and plant traits

Yuki Hayashi, Effect of Bacillus thuringiensis spores on the second stage juveniles of soybean cyst nematode

Dr You-Mie Kim, Target molecules of Bacillus thuringiensis crystal proteins in *C. elegans*

Dr Ayako Kusakabe, Synergistic nematicidal activity of secondary metabolites produced by the entomopathogenic bacterium Photorhabdus l. sonorensis (Enterobacteriacea) against the root knot nematode, Meloidogyne incognita (Nematoda: Tylenchida)

Dr Emilie Lefoulon, Transcriptomic analysis of two entomopathogenic Steinernema nematodes highlights metabolic costs associated with Xenorhabdus endosymbiont carriage

Jayashree Ramakrishnan, Characterization of Entomopathogenic Nematodes at Rapid Desiccation

13:45-14:00 Break

MICROBIAL CONTROL DIVISION SYMPOSIUM: PROMISING MICROBIAL CONTROL OPTIONS FOR FALL ARMYWORM, A GLOBAL PERSPECTIVEECOLOGY (14:00-16:00)

14:00 Roma Gwynn, Michael Brownbridge & Travis Glare An a priori strategy for using market-ready microbial biocontrol products for FAW control: technical, economic and end-user consideration

14:30 Rica Joy Flor Beauveria bassiana for FAW in Cambodia: from on-station experiments to farmer-led experiments and work with non-farmer stakeholders 2017-2020

15:00 Subbi Sevgan A Kenyan and regional perspective of activities on using microbial control agents against FAW: product development and farmer adoption

15:30 Italo Delalibera Progress in Brazil for the control of FAW with microbial pathogens

16:00 - 16h15 Break

CHAT SESSION: ENTOMOPATHOGENIC FUNGI DIVERSITY 1 (16:15-16:45)

Dr Kathryn Bushley, Infection of *Spodoptera frugiperda* with the entomopathogenic fungus *Beauveria bassiana*

Dr Eric Clifton, Diversity of native Hypocrealean fungi infecting the invasive spotted lanternfly in the United States

Ye Ram Im, Management of cotton aphid, Aphis gossypii using entomopathogenic *Beauveria bassiana*

Mika Pagani, Laboratory evaluation of the effectiveness of commercial entomopathogenic strains *Beauveria and Metarhizium* for control of the Cornfield Wireworm (Coleoptera: Elateridae)

Arnaud Segers, Susceptibility of *Bruchus rufimanus Boheman* 1833 (Coleoptera: Chrysomelidae) to three entomopathogenic fungi: Limits of conidial suspension sprayings and pledging alternatives in integrated pest management strategy

Sofia Simeto, Screening of entomopathogenic fungi for virulence against Emerald Ash Borer eggs

CHAT SESSION: ADVANCES IN INSECT MOLECULAR VIROLOGY (16:15-16:45)

Dr Jeff Hodgson, Drosophila as a model to identify viral envelope protein trafficking pathways

Hannah-Isadora Huditz, Identification and Tissue tropism of newly identified iflavirus and negevirus in tsetse flies *Glossina morsitans morsitans*

Xiaoxuan Liu, *Bombyx mori* Pupae Efficiently Produce Recombinant AAV2/HBoV1

Vectors with a Bombyx mori Nuclear Polyhedrosis Virus Expression System

Qingsen Liu, Generation and characterization of the AcMNPV-Bombyx mori bidensovirus chimeras

Remziye Nalçacıoğlu, Functional and Morphological Analysis of Invertebrate Iridescent Virus 6 (IIV6) Potential Matrix Protein (415R)

Fernando Pinheiro Lourenço, Construction of a vector for expression of recombinant proteins in in insect cells' mitochondria

Fernanda Pontes, Equivalence of cypoviruses α-helixes: evidence of convergent evolution of structure and function

Lex van Es, Separating small extracellular vesicles from baculovirus virions

Dr Xi Wang, A functional peroral infectivity complex is present in the envelope of White Spot Syndrome Virus of shrimp

16:45-17:00 Break

VIRUS DIVISION SYMPOSIUM: PROMISING PLACE OF BACULOVIRUSES IN THE FIGHT AGAINST COVID-19 (17:00-19:00)

17:00 Dr Linda King Four decades of the Baculovirus Expression System – from early beginnings to being at the forefront of global efforts against COVID-19

17:35 Gobern Pijlman Two-component nanoparticle vaccine displaying glycosylated spike S1 domain induces neutralizing antibody response against SARS-CoV-2 variants

18:10 Dr Gale Smith Baculovirus-Sf9 Insect Cell Technology in the Development of a COVID-19 Vaccine

18:45 Discussion

19:00-19:15 Break

CHAT SESSION:PATHOGEN PHYSIOLOGY (19:15-19:45)

Haibo Chen The fate of bacteria of the Bacillus cereus group in the amoeba environment

Dr Ekaterina Grizanova Together or separately? Effect of Bacillus thuringiensis spores and Cry toxins on Colorado potato beetle

Loretta Mugo Effect of Diet and Antibiotic on the growth and fitness of laboratory reared Spodoptera exigua (Hübner)

Biko Muita Cellular mechanisms causing midgut damage and insect death upon exposure to Bacillus thuringiensis insecticidal toxin

Dr Ratnasri Pothula Xenorhabdus bovienii strain jolietti requires Type 6 secretion systems to kill closely related bacteria and colonize its nematode host?

Cybele Prigot Immune priming protection against pathogens: what can terrestrial crustaceans tell us about this innate immune ability?

Beatriz Ramírez Serrano Influence of arbuscular mycorrhizal symbiosis and nitrogen levels on the performance of Spodoptera exigua developing on maize: are effects mediated by a change of the insect gut microbiota?

Carlotta Savio Impact of probiotic bacteria on Tenebrio molitor fitness, gut microbial composition and susceptibility to Bacillus thuringiensis serovar tenebrionis and Metarhizium brunneum infections

Jennifer Upfold The role of the microbiota in host resistance to pathogens in Galleria mellonella larvae

CHAT SESSION:ENTOMOPATHOGENIC FUNGI DIVERSITY 2 (19:15-19:45)

Dr Mary Barbercheck Antagonistic effects of endophytic Metarhizium robertsii in maize against the phytopathogen, Cochliobolus heterostrophus

Fabian Garcia Multifunctionality of endophytic entomopathogenic fungi: plant growth promotion and Spodoptera littoralis (Boisduval) (Lepidoptera: Noctuidae) control in melon

Kassie Hollabaugh Identifying Ecological Relationships among Beauveria bassiana and Kudzu Bug, Megacopta cribraria – How Does Seasonality and Endophytic Presence of the Entomopathogen Influence Incidence on Kudzu Bug in East Tennessee?

Naradorn Chui Chai Effect of Induratia fengyangensis volatile compounds on West Indian sweet potato weevil, Euscepes postfasciatus (Fairmaire)

Jana Uthoff Development of seed coatings for Phacelia tanacetifolia with beneficial fungi for plant strengthening and protection against plant parasitic nematodes

Dr Laura Villamizar Characterization and production of Metarhizium majus isolated from coconut rhinoceros beetle in Samoa, Philippines and Malaysia

CHAT SESSION:BACULOVIRUS REPLICATION AND MORPHOGENESIS (19:15-19:45)

Dr Carina Bannach Hyper-expression of baculovirus P10 and processing by viral cathepsin are required for nuclear disintegration and release of polyhedra from Autographa californica multiple nucleopolyhedrovirus-infected cells

Dr Guoqing Chen Autographa californica Multiple Nucleopolyhedrovirus Ac16 modulates the accumulation of IE1

Tong Chen The Autographa californica Multiple Nucleopolyhedrovirus ac26 Gene Is Critical for Morphogenesis of Occlusion Body

Dr Cristina Del Rincón Castro Identification of differential genes in primary infection of Spodoptera frugiperda (Lepidoptera:Noctuidae) with an SfNPV baculovirus

Youpeng Fan Baculovirus Utilizes Cholesterol Transporter NIEMANN–Pick C1 for Host Cell Entry

Dr Guozhong Feng Identification of Spodoptera frugiperda importin alphas that

facilitate the nuclear import of Autographa californica multiple nucleopolyhedrovirus DNA polymerase

Simone Gasque Both the enzymatic- and structural properties of Autographa californica multiple nucleopolyhedrovirus (AcMNPV) protein tyrosine phosphatase (PTP) are insignificant for brain entry in Spodoptera exigua caterpillars

Yue Liu The role of BmNPV Bm65 protein in the repair of ultraviolet-induced DNA damage

Dr Dong Zhanqi BmNPV induces cell cycle arrest and enhances viral replication by depleting BmCDK1 and BmCyclinB

WEDNESDAY 30TH JUNE

CHAT SESSION:PESTICIDAL PROTEIN MODE OF ACTION (13:15-13:45)

Faisal Alzahrani Investigating the importance of Cry2A activation in its activity toward Aedes aegypti

Emily Heath Establishing the role of glycans and lipids in the mechanisms of Tpp1/Tpp2 (Bin) toxin

Dr. Ayda Khorramnejad Is activation of Bacillus thuringiensis Cry1Ia proteins necessary for toxicity?

Daniel Pinos Hetero-oligomerization of Bacillus thuringiensis Cry1A proteins enhance binding to the ABCC2 transporter of Spodoptera exigua

Yudong Quan Specific binding of radiolabeled Vip3Af to brush border membrane vesicles from Spodopterda spp. and determination of the domains involved

CHAT SESSION:PHYSIOLOGICAL INTERACTIONS (13:15-13:45)

Zoltan Erdos Experimental evolution as an approach for increasing virulence in insect pathogenic fungi

Juliana Ferreira Influence of culture medium supplementation on Metarhizium robertsii protease production and response to heat stress

Jéssica Fiorotti Unveiling The Phagocytosis Process In Ixodes Ricinus Challenged By Metarhizium Robertsii

Prof. Jae Su Kim Beauveria bassiana ERL836 and JEF-007 with similar virulence show different gene expression when interacting with cuticles of western flower thrips, Frankniella occidentalis

Dr. Li Ma Secondary metabolites produced by a novel isolate of Metarhizium robertsii (CPD006) during mass production

Ginna Quiroga Kinetic, enzymatic and thermal evaluation of Metarhizium anisopliae conidia produced in solid fermentation

Anna Slowik Quantification of filamentous growth of entomopathogenic fungi using spectrophotometry for rapid and high-throughput analysis

CHAT SESSION:HOST-PATHOGEN INTERACTIONS (13:15-13:45)

Prof. Rollie Clem MicroRNA targeting of Sindbis virus confirms the importance of midgut replication in disseminated infection of Aedes aegypti

Dr. Kayvan Genomic analysis of Oryctes rhinoceros nudivirus (OrNV) and its host, Coconut Rhinoceros Beetle (Oryctes rhinoceros), in South Pacific Islands

Dr. Trevor Jackson Electron microscopy study confirms infection of coconut rhinoceros beetle (CRB-G) gut cells by OrNV V23B

Dr. Jie Li Identification of a PGRP-lb gene in Spodoptera exigua with antiviral function against S. exigua multiple nucleopolyhedrovirus (SeMNPV)

Angel Llopis-Gimenez Baculovirus infection alters olfaction of its lepidopteran host Spodoptera exigua (Hübner, 1808)

Gabriela Maciel Guevara A silent killer of crickets: insights on the transmission of Acheta domesticus densovirus

Annamaria Mattia Investigating the vertical transmission of covert infections by SeMNPV in Spodoptera exigua

Shili Yang Gene expression profiles of different Cydia pomonella granulovirus isolates in midguts of type II resistant coding moth larvae

13:45-14:00 Break

FUNGI DIVISION SYMPOSIUM: PROMISING NEW ADVANCES IN THE WORLD OF THE ENTOMOPHTHORALES (14:00-16:00)

14:00 Dr. Brian Lovett The patient puppetmaster: how Massospora spp. infect and manipulate cicada hosts

14:20 Dr. Carolyn Elya Taking control: Mechanistic insights into the behavioral hijacking of fruit flies by the zombie fungus *Entomophthora muscae*

14:40 Dr. Andreas Naundrup The entomopathogenic fungus *Entomophthora muscae* uses volatiles to fatally attract and trick house fly males to mate with contagious female cadavers

15:00 Dr. Linda Muskat Fermentation and formulation of *Pandora* sp. nov. for biological psyllid pest control

15:20 Dr. Stéphanie Saussure Can fungal epizootics reduce yield loss caused by aphids in cereals?

15:40 Dr. Ann E. Hajek Batkoa major infecting spotted lanternflies: Host range and population structure

16:00-16:15 Break

CHAT SESSION:APPLIED ASPECTS 1 (16:15-16:45)

Jason Bielski Evaluation of different *Beauveria bassiana* GHA formulations against overwintering spotted lanternfly (*Lycorma delicatula*) egg masses with various seasonal applications

Leela Rizal Virulence of field-collected entomopathogenic fungi to diamondback moth larvae – dose, temperature and host starvation effects

Dr. Éverton Fernandes Heat stress causes physical damage on the conidial surface of *Metarhizium anisopliae*

Dr. Drauzio Eduardo Naretto Rangel Fungal tolerance to Congo red, a cell wall integrity stress, as indicator of ecological niche

Shoma Kawa The infection mechanism and dynamics of orally administered *Beauveria pseudobassiana* and toxicity of its secondary metabolites in *Anopheles stephensi*

CHAT SESSION:INSECT MICROSPORIDIA: HOST PATHOLOGY AND DISEASE CONTROL (16:15-16:45)

Dr. Viacheslav Dolgikh Expression of scFv-fragments against Vairimorpha (Nosema) ceranae hexokinase and ATP/ADP carriers suppress microsporidia intracellular development in Sf9 insect cells

Dr. Tamara Gomez-Moracho The gut parasite Nosema ceranae impairs olfactory learning in bumblebees

Inna Grushevaya Nosema pyrausta as natural mortality factor of Ostrinia moths

Dr. Julia Malysh The microsporidium Nosema pyrausta in the beet webworm, Loxostege sticticalis

Dr. Ran Maoshuang Nosema bombycis suppresses host cell apoptosis via Nbserpin14 inhibiting the host Caspase protease BmICE activity

Dr. Dong Zhanqi Silver nanoparticles are effective in controlling microsporidia

CHAT SESSION: ENDOGENOUS VIRUSES (16:15-16:45)

Andrews Alexander Frederic Monvoisin Santos Fisch Induction of apoptosis in insect cells by tyrosine phosphatases from Cotesia flavipes bracovirus

Alexandra Cerqueiro de Araujo Characterization of a new nudiviral endogenization event in the Campopleginae wasp Campoplex capitator

Dr. Kelsey Coffman A viral mutualist employs post-hatch transmission for vertical and horizontal spread among parasitoid wasps

Dr. Maria Cristina Crava Endogenous viral element-derived Piwi-Interacting RNAs (piRNAs): insights from Spodoptera genus

Ming-Wu Dai The fusion of envelopes of Microplitis bicoloratus bracovirus during assembly and invasion

Dr. Jean-Michel Drezen Organization and evolution of endogenous bracovirus in parasitoid wasp genomes

Prof. Elisabeth Huguet Role of endogenized lef-4 and lef-8 nudiviral genes in Virus-Like-Particle production in the parasitoid wasp Venturia canescens

Kelly Tims Effect of Viral RNA Polymerase on Expression of Wasp and Viral Genes in Microplitis demolitor

16:45-17:00 Break

MICROSPORIDIA DIVISION SYMPOSIUM: PROMISING MICROSPORIDIA OF INVERTEBRATE HOSTS IN AQUATIC AND TERRESTRIAL HABITATS (17:00-19:00)

17:00 Prof. Aaron Reinke Comprehensive survey of microsporidia reveals extensive ecological and phenotypic diversity.

17:20 Dr. Yuliya Sokolova Microsporidia in trematodes: an overview and new findings in the USA and Russia

17:40 Dr Jamie Bojko A new microsporidian parasitizing invasive *Carcinus* sp. in the Argentinian Atlantic.

18:00 Dr. Artur Trzebny Specific mosquito gut microbiome members are associated with microsporidian infection.

18:20 Dr Daniela Pilarska A review of research on microsporidia infecting pest insects in Bulgaria.

18:40 Prof Yuri Tokarev How do microsporidia of insect hosts interact with insect parasitoids?

CHAT SESSION: TRANSDISCIPLINARY: INSECT AS FOOD AND FEED AND IN MASS REARING (19:15-19:45)

Dr. Francesco Defilippo Preliminary observations of viral presence in a mass rearing crickets used as feed and food

Dr. Kristin Duffield Identification and quantification of entomopathogenic viruses in reared crickets

Luis Hernández Pelegrín The RNA virome of the medfly: a necessary step to optimize medfly control

Pascal Herren Immune priming in Tenebrio molitor induced by temperature stress and a fungal pathogen

Hannah-Isadora Huditz Identification and Tissue tropism of newly identified iflavirus and negevirus in tsetse flies Glossina morsitans morsitans

Fang Shiang Lim Harnessing the Potential of Real Time Portable Next Generation Sequencing as a Surveillance Tool for Pathogens in Mass Reared Insects

Gabriela Maciel Vergara A silent killer of crickets: insights on the transmission of Acheta domesticus densovirus

Svetlana Malysh Insect iridescent virus type 6 is widespread in wild and cultured insects

Loretta Mugo Effect of Diet and Antibiotic on the growth and fitness of laboratory reared Spodoptera exigua (Hübner)

Robert Pienaar First evidence of long-lasting association between viruses and the Black soldier fly, Hermetia illucens

Dr. Marina Querejeta Coma Drivers and role of bacterial diversity and composition along the developmental stages of the Black Soldier Fly (Hermetia illucens)

Carlotta Savio Impact of probiotic bacteria on Tenebrio molitor fitness, gut microbial composition and susceptibility to Bacillus thuringiensis serovar tenebrionis and Metarhizium brunneum infections

Anna Slowik Quantification of filamentous growth of entomopathogenic fungi using spectrophotometry for rapid and high-throughput analysis

Jennifer Upfold The role of the microbiota in host resistance to pathogens in Galleria mellonella larvae

Dr. Dong Zhanqi Silver nanoparticles are effective in controlling microsporidia

CHAT SESSION: MICROBIAL CONTROL WITH PROTEINS (19:15-19:45)

Dr. Gloria Barrera Cubillos Granulovirus derived proteins (GVPs) to enhance insecticidal activity of Serratia entomophila against grass grub

Juan Carlos Conde Insecticidal action of proteins from the crude extract of Beauveria bassiana on the Mediterranean fruit fly Ceratitis capitata

Rania Jabeur A novel binary pesticidal protein from Chryseobacterium arthrosphaerae controls Diabrotica virgifera virgifera via a different mode of action to existing commercial proteins

Prof. Anant Patel The project Bio-Protect: Target-specific RNA-based bioprotectants for sustainable crop production in a changing climate

Dr. Daniel Sosa-Gómez Outbreaks of Rachiplusia nu (Guenée) in southeastern and southern Brazil are associated with its field resistance to Cry1Ac toxin

Prof. Yutao Xiao Two ABC transporters are differentially involved in the toxicity of two Bacillus thuringiensis Cry1 toxins to the invasive crop-pest Spodoptera frugiperda (J. E. Smith)

THURSDAY 1ST JULY

CHAT SESSION: MICROBIAL CONTROL INTERACTIONS (13:15-13:45)

Dr. Candice Coombes Interaction between indigenous entomopathogenic nematodes and the fungus Metarhizium anisopliae against late instar false codling moth larvae

Pauline Deschodt Mixed pathogen infections and successful transmission: A complex interaction between host pant, timing of infection and pathogen groups

Dr. Carlos Espinel A combined microbial strategy for the biological control of the fall armyworm Spodoptera frugiperda in maize

Dr. Juliana Gomez-Valderrama Effect of interactions among nucleopolyhedrovirus and Metarhizium rileyi on the mortality of Spodoptera frugiperda larvae under laboratory conditions

Dr. Tamryn Marsberg Synergism between a baculovirus and an insect growth regulator?

Dr. Desiree Jakobs-Schoenwandt Innovative formulations for biological plant protection in horticulture

Eleanor Spence Less is More; Improved Control of Trialeurodes vaporariorum by Co-Application of an Entomopathogenic Fungus and an Insect Growth Regulator

Dr. Pilar Vesga Suppressive soil communities as potential insect pest control tools

CHAT SESSION: MICROSPORIDIA BIODIVERSITY AND PHYSIOLOGY (13:15-13:45)

Kai Ehrenbolger Differences in structure and hibernation mechanism highlight diversification of the microsporidian ribosome

Ekaterina Frolova Four microsporidian hyperparasites of the bristle worm Pygospio elegans

Dr. Nadezhda Isakova Occurence of microsporidia in trematodes infecting snails in St. Petersburg (Russia) water basins

Darya Kireeva Genetic diversity of microsporidia from lepidopteran insects in Russia and neighboring countries

Anastasiya Kononchuk Novel findings of Microsporidia in predatory mites

Arina Rumiantseva Susceptibility of beet webworm larvae to microsporidia from Lepidoptera

CHAT SESSION: VIRUS DETECTION AND IDENTIFICATION (13:15-13:45)

Dr. Francesco Defilippo Preliminary observations of viral presence in a mass rearing crickets used as feed and food

Luis Hernández Pelegrín The RNA virome of the medfly: a necessary step to optimize medfly control

Fang Shiang Lim Harnessing the Potential of Real Time Portable Next Generation Sequencing as a Surveillance Tool for Pathogens in Mass Reared Insects

Svetlana Malysh Insect iridescent virus type 6 is widespread in wild and cultured insects

José Luis Duarte de Jesús Insect and plant virus diversity associated with the vine mealybug Planococcus ficus

Adrià Mengual-Martí Compatibility of covert infections with RNA viruses with natural enemies in Spodoptera exigua

Dr. Madoka Nakai Oryctes rhinoceros nudivirus infections of G-haplotype coconut rhinoceros beetles (Oryctes rhinoceros) in Palauan PCR-positive populations

13:45-14:00 Break

DISEASES OF BENEFICIAL INVERTEBRATES & VIRUS CROSS-DIVISION SYMPOSIUM: VIRUSES OF POLLINATORS (14:00-16:00)

14:00 Dr. Adam G. Dolezal Combined impacts of virus and nutrition on honey bee health

14:30 Dr. Eugene Ryabov DWV/VDV1 infectious clones and their application for study of bee-virus interactions.

15:00 Dr. Ya Guo Virus-blocking peptides to mitigate virus burden in the honey bee.

15:30 Dr. Lena Wilfert Comparative virus population genetics in *A. mellifera* and *A. cerana* in Asia.

16:00-16:15 Break

CHAT SESSION: RECEPTORS AND RESISTANCE (16:15-16:45)

Ascensión Andrés Garrido Cadherin fragment from Spodoptera exigua enhances Cry1A toxicity to Grapholita molesta

Rey Cotto-Rivera Bt resistance-associated alteration of aminopeptidase N (APN) gene expression is independent of the ABCC2 gene in Trichoplusia ni

Prof. Davis Heckel Identification of a new Cry1Ac resistance gene in Heliothis virescens

Dr. Patricia Hernández-Martínez Comparison of in vitro and in vivo binding sites competition of Bacillus thuringiensis Cry1 proteins in two important corn pests

Maria Lázaro-Berenguer In vivo competition assays between Vip3 proteins confirms the existence of shared binding sites among them in Spodoptera littoralis with different relevance on the toxicity

Dr. Mark Nelson Functional validation of DvABCB1 as a receptor of Cry3 toxins in western corn rootworm, Diabrotica virgifera virgifera

Daniel Pinos Alteration of a Cry1A shared binding site in a laboratory selected strain of Ostrinia furnacalis resistant to Cry1A proteins

Dr. Ping Wang Resistance to Bt Cry1Ac in Trichoplusia ni is conferred by multiple gene mutations

Wang Yonghao The Silkworm ABCC transporters are involved in susceptibility difference for each Bacillus thuringiensis Cry1Ab, Cry1Ac and Cry1Fa toxin

CHAT SESSION: APPLIED ASPECTS 2 (16:15-16:45)

Ayaovi Agbessenou Making the right decision: Temperature-dependent modelling approach and spatial prediction reveal suitable areas for deployment of two Metarhizium anisopliae isolates for sustainable management of Tuta absoluta

Dr. Maria Fernandez-Bravo Effect of natural occurrence of Metarhizium spp. on soil arthropod communities in three permanent grassland plots in Switzerland

Steffan Hansen Virulence and natural associations of entomopathogens with adults of the cryptic Phlyctinus callosus species complex

Dr. Dana Ment Not only a formulation: The effects of Pickering emulsion on the entomopathogenic action of Metarhizium brunneum

Dr. CM Senthil Kumar Field evaluation of Akanthomyces (=Lecanicillium) psalliotae and development of an Integrated Pest Management strategy against cardamom thrips, Sciothrips cardamomi

Dr. Shaohui Wu Post-application persistence and field efficacy of a new strain of Cordyceps javanica against the silverleaf whitefly, Bemisia tabaci biotype

So Eun Park Biopesticide using Entomopathogenic fungi Beauveria bassiana Entomopathogenic fungi-mediated management in field

CHAT SESSION: ADVANCES IN FORMULATION, APPLICATION AND CONTROL OF PESTS (16:15-16:45)

Murray Dunn Optimisation of the in vitro liquid culture process of Steinernema yirgalamense and Steinernema jeffreyense using local resources for cost-effective production

María del Mar González-Trujillo Screening of adjuvants to enhance the entomopathogenic nematode survival and adherence after aerial application on grapevine leaves

Dr. Ivan Hiltpold Potential of entomopathogenic nematodes to mitigate the insect vector of the Syndrome de Basse Richesse in sugar beet

Prof. Nona Mikaia Potential of entomopathogenic nematode isolates from Germany and Israel to control the tomato leaf miner(Tuta absoluta, Meyrick) (Lepidoptera:Gelechiidae) in Georgia

Dr. Jaime Ruiz-Vega Performance of Steinernema glaseri pre-conditioned IJs formulated as pellets with sodium poliacrilate

Dr. Ramandeep Kaur Sandhi Entomopathogenic nematodes applied as infected Galleria mellonella cadavers against wireworms (Coleoptera: Elateridae)

Dr. David Shapiro-Ilan Biocontrol with Benefits: Control of Peachtree Borer with Entomopathogenic Nematodes

Ignacio Vicente-Díez Steinernema carpocapsae and Xenorhabdus nematophila based products for the control of the grapevine moth and the grey mold in vineyards

CHAT SESSION: VIRAL BIOINSECTICIDE (16:15-16:45)

Dr. Paola Emilia Cuartas Bio-Insecticidal potential of alphabaculovirus and betabaculovirus mixtures to control the Fall Armyworm Spodoptera frugiperda (J.E. Smith, 1797) (Lepidoptera: Noctuidae)

Dr. Cristina Del Rincón-Castro Characterization of native Mexican strains of baculovirus with virulence towards Spodoptera frugiperda (Lepidoptera:Noctuidae)

Dr. Robert Harrison Insecticidal properties of isolates of Spodoptera frugiperda multiple nucleopolyhedrovirus (SfMNPV) against corn- and rice-strain Spodoptera frugiperda larvae, and genome analysis of selected SfMNPV isolates

Dr. Aurélie Hinsberger Multiple baculovirus infections in codling moth: CpGV-R5

help to CpGV-M cannot be substituted by CrpeNPV
Dr. Aurélie Hinsberger Mixed infections of type I resistant codling moth larvae in treated orchard leaves
Christian Oehlmann Amplicon-based sequence analyses of single nucleotide polymorphisms reveal the genetic structure of LdMNPV field populations
Sofian Renoult Resistance of Cydia pomonella to all viral isolates used in biological control in Europe
Marcel van der Merwe Yeast-baculovirus synergism for the improved control of Thaumatotibia leucotreta, an important pest of citrus in Africa

16:45-17:00 Break

DISEASES OF BENEFICIAL INVERTEBRATES & VIRUS CROSS-DIVISION SYMPOSIUM: VIRUSES OF POLLINATORS (17:00-19:00)

17:00 Dr. Patricia Casino Mechanism of action of Vip3 proteins inferred from their structures

17:30 . Dr. Neil Crickmore Pesticidal protein mechanism of action – the importance of experimental verification

17:40 Dr. Alejandra Bravo Experimental evidence for Cry protein MoA models

18:10 Dr. Mario Soberón The activity of Cry protoxins.

18:40 General Discussion

19:00-19:15 Break

CHAT SESSION : STRAINS AND PROTEINS (19:15-19:45)

Yudong Quan The rapid evolution of resistance to Vip3Aa insecticidal protein in Mythimna separata (Walker) is not related to altered binding to midgut receptors
Dr. Rahul Banerjee Peptide mediated enhancement of a bacterial ETX-MTX pesticidal protein for suppression of the southern green stink bug, Nezara viridula
Yang Geng A novel insecticidal protein is toxin to Ostrinia furnacalis and Agrotis ípsilonfrugiperda larvae, and genome analysis of selected SfMNPV isolates
Rim Hamze Pseudomonas protegens as a biocidal agent against Diptera of medical-veterinary importance
Dr. Jorge E. Ibarra Occurrence of endophytic Bacillus thuringiensis strains in wild vegetation plants
Lainey Williamson Crystal Structure of Lysinibacillus sphaericus Tpp49 using Serial Femtosecond Crystallography
Dr. Jorge E. Ibarra Effect of the Cry10Aa protein from Bacillus thuringiensis expressed in Coffea arabica plants on the coffee berry borer (Hipothenemus hampei)

Dr. Ruchir Mishra Streamlined phage display library protocols for identification of insect gut binding peptides highlight peptide specificity

CHAT SESSION : MICROBIAL CONTROL WITH FUNGI (19:15-19:45)

Nushrat Harun Antara Chitin amended media: A solution for improved entomopathogenic fungi against codling moth
Dr. Inmaculada Garrido-Jurado The ingestion of Metarhizium-colonized plants produces direct and indirect effects on the cotton leafworm Spodoptera littoralis
Anthony George Impact of tannins from bioactive plants on the growth and spore production of the biocontrol fungus Duddingtonia flagrans
Valesca Lima Tick cuticle lipids may limit infection by entomopathogenic fungi
Chiara Pedrazzini What is the effect of geographic and temporal separation of the Common cockchafer on the population structure of its main fungal pathogen?
Cárita de Souza Ribeiro-Silva Conidial production from granules of Metarhizium humberi microsclerotia on soil samples
Antonia Romero Influence of abiotic factors on the persistence and viability of microsclerotia produced by the entomopathogenic fungus Metarhizium spp. (Hypocreales: Clavicipitaceae)
Dr. Prakya Sreerama Kumar A mycelial–conidial formulation of a silkworm-safe isolate of Hirsutella thompsonii to control Polyphagotarsonemus latus in mulberry
Maria Zottele Digging into the past: Metarhizium brunneum as control agent against the sugar beet weevil (Asproparthenis punctiventris)

FRIDAY 2ND JULY

NEMATODE DIVISION SYMPOSIUM: ENTOMOPATHOGENIC NEMATODES OR SCAVENGERS: REVISITING THE EMERGING NEW NEMATODES CLASSIFIED AS EPN (13:30-15:30)

13:30 Dr. Ernesto San-Blas Scavenging among entomopathogenic nematode species: Are there better performers?

13:50 Dr. Vladimir Puza Entomopathogenicity and scavenging behaviour of *Oscheius* nematodes and their competition with entomopathogenic nematodes

14:10 Dr. Giulia Torrini The enigmatic status of *Oscheius onirici* (Nematoda: Rabditida).

14:30 Dr. Javad Karimi Biological and taxonomic characterization of a superior infective isolate of Acrobeloids spp.

14:50 Dr. Raquel Campos-Herrera The cost of fighting for surviving in a complex world: entomopathogenic nematodes as scavengers

15:10 Kyle Anesko Old and new examples of nematodes classified as EPNs

15:30-15:45 Break

PLENARY SYMPOSIUM: SIP AWARDEE SYMPOSIUM (15:45-17:15)

15:45 Vera Ros Welcome and honoring Martignoni Awardee

15:50 Presentation Mauro Martignoni Awardee
Hiroyuki Hikida bv/odv-e26 is required for virus-induced host behavioral manipulation in lepidopteran nucleopolyhedroviruses

16:10 Dr Johannes Jehlei Laudatio of Early Carreer Awardee 2020 Jörg Wennmann

16:40 Dr Jorg Wennmann Deciphering the population structure of baculoviruses by nucleotide polymorphisms

16:45 Dr Patricia Golo Fungi for tick control: what do we know and what do we need to know?

17:15-17:30 Break

PLENARY SYMPOSIUM: SIP BUSINESS MEETING (17:30-18:30)

18:30 Meeting Closure

SOMMAIRE

CONVENORS

Dr Elisabeth Herniou

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Dr Elisabeth Herniou obtained her Master in Evolutionary Biology from the University of Paris-Saclay (France). She then worked at the Natural History Museum in London (UK). Afterwards, she obtained her PhD at Imperial College London (UK) for her work on the genomics and evolution of baculoviruses. She then pursued with a postdoc and Royal Society Fellowship in the UK before joining the CNRS at the Insect Biology Research Institute (IRBI) based at the University of Tours, France. She currently holds CNRS Research Director position. Her research aims at deciphering how biological interactions between insect and viruses impact genome evolution and ecological adaptation. This leads her to study insect and viral biodiversity, towards applications in biological control and insect as food and feed.

Dr María-Cristina Del Rincon-Castro

LE STUDIUM / Marie Skłodowska-Curie Research Fellow

Food and Plant Biotechnology Laboratory, University of Guanajuato - MX

Lascuráin de Retana No. 5, Col. Centro C.P. 36000
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Dr. Cristina Del Rincón obtained her Master and PhD in Plan Biotecnhology from the Center for Research and Advanced Studies (CINVESTAV) (México) for her work in the study and molecular characterization of baculovirus strains for the control of pest insects of agricultural importance. Currently is a Full Professor and was head of Master and Doctoral School in Biosciencies for 10 years, in the Food Department at the University of Guanajuato in México. She is a member of the National System of Researchers (Level 2) and Member of the National Academy of Sciences; was President of the Mexican Society of Biological Control. She is focused on the characterization, identification, molecular biology, and evaluation of entomopathogenic viruses as biological control agents of pests and as expression vectors of eukaryotic genes for 30 years. She has published more than 50 research articles in international journals and 10 books chapter. She has been responsible for 17 research grants related to biological control agents for pests of economically important insects in México.

KEYNOTE SPEAKERS

PLENARY SYMPOSIUM : CURRENT CHALLENGES FOR THE MICROBIAL CONTROL OF SPODOPTERA FRUGIPERDA

Dr Buyung Hadi

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Buyung Hadi serves as an Agricultural Officer (IPM) in Pest and Pesticide Management Team under FAO's Plant Production and Protection Division. He also coordinates the secretariat for the Global Action for Fall Armyworm Control. His professional interests include development of IPM strategies across multiple crops and agroecosystems and creation of the enabling socio-economic and policy environments for successful scaling up of the strategies.

How not to waste a crisis: A pest invasion as an opportunity to scale up biopesticides

Fall armyworm is considered one of the ten worst invasive pests and diseases threatening global food security and livelihoods. It has the potential to destroy up to 80 million tonnes of maize worth USD 18 billion per year in Africa, Asia and the Near East.

Since its first report outside the Americas in 2017, the pest has now been reported in over 70 countries in these regions. In 16 of these countries, there are 26 million people suffering from highly acute food insecurity and invasive pests such as the fall armyworm is one of the main drivers for the insecurity.

In many countries, pesticides, including highly hazardous ones, are used as the primary management tool against the invasive pest, increasing health risks for farmers and their family members. Biopesticides form a relatively small but promising portion of the management tools against fall armyworm. It is thus important to address the bottlenecks to scale up biopesticide use among smallholder farmers in the wake of fall armyworm invasion. Some bottlenecks and roadblocks as well as opportunities for biopesticide scale-up will be discussed.

Dr Emmanuelle d'Alençon

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Emmanuelle d'Alençon is research director at the Diversity, Genomes, Insect Microorganisms Interactions Laboratory (DGIMI), a joint laboratory between INRAE & University of Montpellier, where she is group leader of the team "Epigenetics, Holocentrism, Adaptation" (EHA), working on genome structure and evolution in phytophagous insects. Emmanuelle d'Alençon received her PhD in 1993 at the University of Paris XI for studies having led to demonstration of the copy-choice illegitimate DNA recombination mechanism in model bacteria. She has been working for three years during her post-doc at the Institut Jacques Monod in Paris in Dr M. Kohiyama's laboratory on regulation of chromosomal replication in E. Coli. Interested in mechanisms and forces shaping the genomes, she then joined Dr Philippe Fournier's lab to work on holocentric chromosome structure and evolution in the Lepidoptera. Her group exploits recent advances in lab and pop genomics, transcriptomics, epigenomics and post-genomics functional tools to infer molecular changes at play during adaptation and speciation in Lepidoptera. She is responsible for animation of ADALEP, a INRAE network of 26 french labs working on adaptation in Lepidoptera (https://www6.inrae.fr/adalep). She led the International Public Consortium for the whole genome sequence of S. frugiperda until its publication in 2017.

Know your enemy: Integrative study of plasticity, adaptive evolution and speciation in the Fall armyworm.

Populations have to keep their ability to survive and reproduce to be maintained upon environmental changes. This is challenging especially when fast changes occur and require adoption of new features, either metabolic, developmental, behavioral, physiological or morphological providing an enhanced fitness, through the process of adaptation. Two main mechanisms of adaptation have been described "phenotypic plasticity" and "adaptive evolution". Phenotypic plasticity is defined as the ability of organisms to change of phenotype without changing genotype in response to environmental conditions [1]. Since they do not involve mutations, these different phenotypes are expected to involve distinct transcriptional programs. In the case of adaptive evolution, populations can acquire a better fitted phenotype to the environment thanks to the spread in the population of pre-existing genetic variants or of new mutations conferring enhanced capability (For review, [2] and [3]). Furthermore, adaptation to a novel environment can lead to evolution of reproductive barriers between populations and to the process of ecological speciation [4].

Phytophagous insects are particularly relevant models for the study of plasticity, adaptive evolution and speciation. Their dependence on their host plants for food, oviposition site, refuge against predators and sometimes for reproduction in addition to the parallel evolution of plants in response to this herbivorous pressure lead to constant adaptive changes in their physiology and behavior. Fidelity to their host-plants can promote spatial or temporal isolation of insect populations leading to new species [5].

Our main insect model, the moth Spodoptera frugiperda, or "fall armyworm (FAW)" is a pest of crops. It exists as two variants, one found on corn (C strain), the other found on rice or pasture grass (R strain). Both variants coexist in the same geographical areas, initially America, and are morphologically indistinguishable but possible to identify genetically. Since 2016, it has become invasive in Africa, India, China, Australia and threats European agricultures.

Despite of this apparent preference for different host-plant ranges, the two strains remain highly polyphagous. By a combination of life history traits measurements and omics approaches, we explored the molecular basis of this plasticity. Using comparative transcriptomics and genomics approaches, we also analyzed the molecular basis of adaptive evolution to different host-plant ranges in lab and natural populations. By population genomics approaches, we have started to characterize the genomic signatures of adaptive evolution and strain differentiation, both in native and invasive populations. I will present our current hypotheses on the evolutionary status of the Fall armyworm based on this integrative study.

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Julio studied entomology and plant pathology at Mexico's Universidad Autónoma Chapingo, and subsequently obtained MS and PhD degrees at University of California, Riverside. He currently is Professor at Texas A&M University, where he has taught courses in biological control, host plant resistance, integrated pest management, and entomology. His research has focused on three broad areas: biological control, parasitoid ecology and behaviour, and herbivore defence evolution. In the latter area, his research has sought to shed light on how herbivore defences have evolved in maize in the contexts of crop domestication, spread, and breeding. Recently, this research has expanded to include maize microbiome-associated herbivore defences, including how they may be used for biological control.

Reclaiming an ancestor's legacy: Fortifying the maize microbiome against fall armyworm herbivory using teosinte microbiota

Our recent research explores plant microbiome contributions to maize defence against pests, particularly fall armyworm (FAW). It addresses questions stemming from ecological inferences of hologenome evolution, and their implications for applied bioprospecting, e.g.: how the plant microbiome is inherited between generations in the maize ancestor teosinte; whether such hologenomic inheritance is affected by domestication and breeding; whether microbiome-derived defenses can be transplanted from teosinte to maize to improve pest management, and implications for other crops. We present results concerning the latter of these questions: Whether inoculums made from teosinte leaf litter and microbial enrichment can enhance FAW resistance in maize. We tested a leaf litter inoculum consisting of an aqueous suspension of senesced teosinte leaves, and a microbial enrichment inoculum derived from teosinte green leaf tissue. Both treatments enhanced the resistance of maize seedlings to FAW, and rendered the seedlings less attractive to FAW larvae. Additionally, we are developing tools to handle mixed microbial enrichments for developing bioactive inoculums. Firstly, we designed a high-throughput method for microbial isolation that allows simultaneous culture of diverse microorganisms. And, we are surveying actinomycetes diversity in teosinte, as these bacteria are keystone taxa and can potentially allow microbiome transplantation using simplified, less diverse microbial inoculums.

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Following my PhD (Imperial College), I spent 4 years as a post-doc in Jenny Cory's group at the Institute of Virology & Environmental Microbiology (Oxford) working on iridescent viruses. In 1994, I moved to southern Mexico and began working on control of S. frugiperda, thanks to a European Union funded project (1997) that was the start of a formal collaboration with Primitivo Caballero in Pamplona, Spain. After moving to Spain in 2002, I worked alongside Primitivo and his students, including Oihane Simón and Rosa Murillo, on the insecticidal properties of SeMNPV and SfMNPV. In 2006 I returned to Mexico and began work at the Instituto de Ecología AC, since when I have continued to collaborate with Primitivo and his group and also work on other aspects of biorational

Two decades of collaborative research on Spodoptera frugiperda MNPV.

Over two decades of collaboration involving the group of Primitivo Caballero (IMAB-UPNA, Spain), Miguel Lopez-Ferber (IMT Mines Alès, France) and myself (INECOL, Mexico) have focused on the genomic characteristics, genetic structure, genotype interactions, ecology and insecticidal characteristics of Spodoptera frugiperda multiple nucleopolyhedrovirus (SfMNPV). These studies have generated several unexpected findings, including the beneficial role of defective genotypic variants on virus infectivity and the transmission of genotypic diversity. They have also provided two novel paradigms for the design of virus insecticides. The first of these involves testing, selecting, mixing and passaging known proportions of different genotypic variants to produce unique combinations of variants with improved insecticidal properties. For the second, we used SfMNPV to demonstrate the production of co-occluded mixed virus preparations, and how superinfection exclusion can influence the composition of virus progeny that result from the sequential inoculation of host insects. Finally, I will mention recent studies on the production of SfMNPV occlusion bodies (OB) and the role of OB maturation on the insecticidal activity of OBs harvested from living and virus-killed larvae. It has only been possible to generate these advances through a series of collaborative studies involving students and postdocs and most recently, the university spin-off Bioinsectis.

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Dr Popham received her PhD in insect physiology from the University of Missouri (USA) following which she was a postdoctoral associate at the University of Georgia with Louis Miller characterizing baculovirus genes that impact potency on lepidopteran pests. Afterward she was a Research Biologist with the USDA Forest Service in Ohio, with Jim Slavicek studying genes that impact the infectivity or speed of the gypsy moth baculovirus. In 2002 she accepted a position as Research Scientist with USDA Agricultural Research Service, in Missouri, to research the insect immune response to baculoviruses and characterize baculoviruses. In 2013 she became Chief Scientist for AgBiTech, headquartered in Texas, that manufactures and sells baculoviruses for agricultural use.

How SfMNPV has moved from a concept to a control method of *Spodoptera frugiperda*

As challenges to control insect pests increase due to chemical insecticide resistance, the need and interest to develop alternative control methods has accelerated in recent years. Baculoviruses have long been known to provide lepidopteran pest control in various crops but historically, there has been limited investment in high quality formulations and reliable processes for large scale manufacturing. Regulatory requirements are evolving as regulators strive to understand and adapt to the critical differences between chemical vs. biological products. Registration processes vary from relatively simple in same countries to highly prolonged and inefficient in others. Increased understanding of the mode of action (MOA) of baculovirus through identification of the *per os* infectivity factors has allowed the approval of the new Group 31 to the Insecticide Resistance Action Committee (IRAC) MOA classification scheme. Recognition of this product category as a valuable tool for insecticide resistance management encourages growers to incorporate baculovirus-based insecticides as a relevant component in their management programs. With the rapid spread of *Spodoptera frugiperda* into new continents and crops, the global interest in *Spodoptera frugiperda multiple nucleopolyhedrovirus* (SfMNPV) as a management tool has increased significantly. A discussion of the challenges and successes in developing, registering, manufacturing and launching baculovirus-based insecticides will be presented.

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Professor (Dr) Jurat-Fuentes obtained his BS in Biology and a MS in Genetics from the University of Valencia (Spain). He obtained his PD in Entomology at the University of Georgia (USA). He joined the faculty in the Department of Entomology and Plant Pathology at the University of Tennessee. He is currently Professor in the area of Insect Physiology and Molecular Pathology. His research aims at developing efficacious insecticidal technologies for more sustainable and safer food production. He has extensive expertise on the mode of action and resistance mechanisms against insecticidal proteins from Bacillus thuringiensis and insecticidal gene silencing by RNA interference (RNAi). He has authored >80 peer-reviewed publications and 13 book chapters, and has received multiple research awards.

Challenges and opportunities for bacterial control of *Spodoptera frugiperda*.

The fall armyworm (*Spodoptera frugiperda*) is quickly becoming a global super pest affecting numerous food and fibre staple crops. In its native range, larvae of fall armyworm have been controlled using insecticidal proteins (IPs) from bacteria, mainly *Bacillus thuringiensis* (Bt). In addition to bacterial pesticides, effective fall armyworm control has been provided by Cry1F, Cry1Ab, Cry1A.105, Cry2Ab and Vip3Aa Bt proteins produced as plant incorporated protectants in corn, cotton and soybean. However, the evolution of practical resistance threatens sustainability of fall armyworm control with these bacterial IPs. In the past decade, field resistance to Cry1F, Cry1Ab and Cry1A.105 proteins has been reported for *S. frugiperda* populations in Puerto Rico, south-eastern USA (Florida and North Carolina), Brazil and Argentina. This resistance is recessive and genetically linked to mutations in an ABC transporter superfamily C2 gene (SfABCC2) that serves as Cry protein receptor. Major alleles of resistance to Cry2 and Vip3Aa IPs have also been detected in field S. frugiperda populations. The long range migratory behaviour of S. frugiperda and its ever expanding invasive range suggest the risk of long range spread of resistance alleles to bacterial IPs. This presentation will review up-to-date knowledge on resistance to bacterial IPs, implications of migration for resistance spread, and emerging opportunities for bacterial control of fall armyworm populations.

DISEASES OF BENEFICIAL INVERTEBRATES DIVISIONAL SYMPOSIUM : PATHOLOGICAL ADVANCES IN CARCINOLOGY

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Dr. Kough grew up in Washington, DC and in the state of Maryland, famous for its exceptional crab cakes which he eats with great gusto. Andy earned a BS from landlocked Gettysburg College before pursuing a PhD in Marine Biology at the University of Miami focused on Caribbean Spiny Lobster larval transport. He has published on how animal movement informs conservation management for spiny lobster, queen conch, grouper, snappers, and on freshwater lake sturgeon.

Disease slows crawling crabs and alters modeled connectivity between North American *Callinectes* sapidus populations

Co-authors : Kough, Andrew S.[1]; Behringer, Donald C.[2,3]; Bojko, Jamie[3,4]; Plough, Louis5; Schott, Eric[6]

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Blue crabs (Callinectes sapidus) are a feisty generalist species that inhabits a large swath of coastal habitat from New England down to Argentina. Throughout this range they thrive within variable environmental conditions and life history including changes in phenology and temperature-based dormancy. In addition, the virus CsRV1 infects them and can lead to higher mortality. However, one sublethal effect may be a decrease in adult mobility which in turn reduces crab dispersal potential. Our goal was to quantify the scales of adult dispersal in crabs to estimate the impact of disease on demographic connectivity. We tracked unconstrained adult crabs offshore to inform a biophysical model of adult crab dispersal. In addition, we used laboratory raceway experiments on healthy and clinically infected crabs to estimate sublethal impacts on crab mobility. Our results show how changing life history and disease drive the dispersal and connectivity of blue crabs in the USA across a climatic gradient from subtropical Gulf of Mexico waters through seasonally dormant populations in Maine. Our dispersal model suggested that scuttling adults could cross local oceanographic boundaries and keep the population connected, resilient, and replenished. However, disease reduces crab population connectivity and may therefore have greater impacts on the species than mortality alone would suggest.

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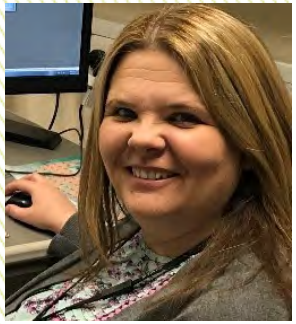
Elizabeth (Liz) Duermit-Moreau is a fourth year PhD Candidate in Fisheries and Aquatic Sciences at the University of Florida. She received her M.S. in Marine Biology at the University of Charleston, South Carolina, and her B.S. in Zoology from Michigan State University. For her dissertation she is researching how fisheries and habitat degradation impact disease ecology of decapod crustaceans. Outside of research Liz enjoys hiking with her dog, kayaking, and gardening.

Diversity and disease of mobile benthic fauna in Florida Bay after harmful cyanobacteria blooms degrade hard-bottom habitat

Shallow hard-bottom habitat covers ~30% of Florida Bay and is designated as an Essential Fish Habitat for commercially fished teleosts and invertebrates. It is dominated by a diversity of sponges, which give structural complexity to the otherwise low-relief habitat. This habitat and its many ecosystem functions can become severely impaired after periodic cyanobacterial blooms. The direct and indirect effects of these blooms on the commercially important Florida stone crab *Menippe mercenaria* and Caribbean spiny lobster *Panulirus argus*, and their diseases, remain broadly unknown. These species are affected directly by habitat loss (sponge die-off), but indirect effects due to potential lack of prey and upon their local epidemiology remain understudied. In the summer of 2019, we surveyed three healthy sites and three sites degraded by blooms in Florida Bay. We used transects to quantify the structural differences across the habitats and collected benthic fauna by suction sampling to explore changes in biodiversity. Up to 30 M. mercenaria and P. argus were collected from each site and were screened to discern their pathogen profiles. The results include the detection of '*Panulirus argus Mininucleovirus*' in P. *argus* and a *trophically* transmitted gregarine in *M. mercenaria* that may use local fauna to transmit. This study increases our understanding of the ongoing changes in Florida Bay from epidemiological and biodiversity perspectives in response to cyanobacterial blooms.



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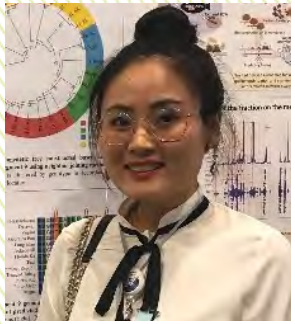
Dr Kelly Bateman is an Invertebrate Pathologist at the Centre for Environment, Fisheries and Aquaculture Science (Cefas) in the UK. Her work focuses on aquatic pathogens, and combines approaches based upon histopathology, transmission electron microscopy and molecular systematics for the classification of novel and emerging pathogens. Kelly is leading the work of the Crustacean Health Theme within the OIE Collaborating Centre for Emerging Aquatic Animal Diseases at Cefas. She has over 20 years' experience in the diagnosis of disease in experimental, farmed and wild aquatic animals, with a special interest in the identification and characterisation of novel and emerging pathogens of crustaceans. Kelly is an author on over 50 peer-reviewed publications in the field of aquatic animal health.

Emergence of paramoebiasis in edible crabs (*Cancer pagurus*) from UK waters

Co-Authors: Stentiford, G.D., Kerr, R., Stone, D., Feist, S.W., White P., Edwards, M. Green, M.J., Ross, S., Evans, C., Bass, D.

The genus *Paramoeba* (including *Neoparamoeba*) (Amoebozoa, Dactylopodida) includes well known opportunistic pathogens associated with fish (*N. peruans*; amoebic gill disease), lobsters, molluscs, and sea urchins, but only rarely with crabs (grey crab disease of blue crabs). Following reports of elevated post-capture mortality in edible crabs (*Cancer pagurus*) captured from parts of the English Channel fishery in the UK, a novel disease (paramoebiasis) was detected in significant proportions of the catch. We present histopathological, transmission electron microscopy, and molecular phylogenetic data showing that this disease was associated with infection by a novel *Paramoeba* spp. The disease was defined by colonization of haemolymph, connective tissues and fixed phagocytes by amoeboid cells, leading to tissue destruction and presumably, death in severely diseased hosts. Analysis of phylogenetic data highlighted the condition is associated with a novel Paramoeba lineage, described herein for the first time. Novel Paramoeba lineage is shown to be morphologically similar but genetically distinct from other members of the genus. We name the novel parasite *Paramoeba feisti* n. sp. and highlight the emergence of paramoebiasis in *C. pagurus* from UK waters.



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I graduated from Ocean University of China as an undergraduate student in 2014 and got my master's degree in the Institute of Oceanology, Chinese Academy of Sciences in 2017. I studied on neutrophil extracellular traps - its production and anti-bacteria functions in tongue sole for my master's thesis. Now I am a PhD candidate in University of Maryland, Baltimore County, working in Institute of Marine and Environmental Technology on virus diseases in blue crab. My PhD research project focuses on identifying the virome of blue crab and investigating interactions between the pathogenic virus and host life history. In the future, I will seek postdoc opportunities to work on virus discovery and characterization as well as interactions between virus infections and host immune response.

A widely distributed pathogenic reovirus affecting the Atlantic blue crab, *Callinectes sapidus*

Callinectes sapidus, an important species native to the Atlantic coast but invasive to the Mediterranean, has a broad distribution spanning tropical and temperate climates. Its life history varies latitudinally, from extended overwintering at high latitudes to year-round activity in tropical locations. *Callinectes sapidus* reovirus 1 (CsRV1) is a pathogenic virus causing severe disease and mortality in *C. sapidus*. This study used Rt-qPCR to investigate CsRV1 prevalence in *C. sapidus* and *Callinectes* spp. across latitudinal shifts in climate and crab life history. CsRV1 was present at a wide range of geographic locations, including the North and South Atlantic coasts, the Caribbean Sea and the Gulf of Mexico. CsRV1 prevalence in *C. sapidus* was significantly higher at temperate than tropical latitudes but not detected in other Callinectes spp. CsRV1 genome is composed of 12 segmented dsRNA and has high nucleotide sequence identity (>98%) with 'P virus' found infecting Macropipus depurator in the Mediterranean. This suggests they are likely variants of a single reovirus species that infects different hosts on different continents. An investigation of CsRV1 in *C. sapidus*, and other crab species in the Mediterranean will help us understand the origins of this virus, the direction of its trans-Atlantic movement, and whether it was carried by *C. sapidus* or an unknown crustacean species. These findings expand our understanding of how oceanic connectivity and host life history can drive the distribution of marine pathogens, and may potentially offer further insights into the role of pathogens in mitigating or facilitating invasions.

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I am currently a Fisheries Research Officer at Swansea University where my research focuses on diseases in marine ecosystems, which have the potential to alter ocean life, and the coastal communities that rely upon it. I obtained my BSc in Biology and PhD at Swansea University, studying pathogens of economically important European shellfish. My first postdoctoral position at the National Autonomous University of Mexico's Institute of Marine Sciences and Limnology investigated the relationship between tropical habitat ecology and disease prevalence in the Caribbean spiny lobster. My current research, through the BlueFish Project, investigates disease connectivity in the aquatic environment, including the ability to 'track' pathogens from the water column into the host using eDNA approaches.

Disease connectivity: Investigating disease dynamics in shore crabs, *Carcinus maenas*

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The green, or common shore crab, Carcinus maenas, although native to Britain and Ireland has been introduced to the USA, Sri Lanka, Red Sea, Madagascar, South Africa and Australia. This species hosts a range of micro- and macro-parasites, including the dinoflagellate, Hematodinium spp. Due to its introduction to a wide range of areas and sharing of habitats with species of commercial importance, it is an important species in which to monitor disease.

Crabs (n=50/location) and water (2L/location) were sampled monthly from two distinct locations; a closed Dock and an intertidal Pier, over 12 months. Molecular screening of both crab DNA and water eDNA, in addition to histological screening of crab gills and hepatopancreas for Hematodinium spp. and co-infections took place.

Overall, 13.6% of crabs were *Hematodinium* spp. positive from PCR analyses (14.4% Dock and 12.8% Pier location) with significant seasonal patterns in the Pier location. Males were more likely to host *Hematodinium* sp. than females overall and in the Dock location, but not in the Pier. Size was a significant factor in determining the disease in the Pier location only, where crabs presenting Hematodinium sp. were significantly smaller. *Hematodinium* spp. were found in eDNA of just the Pier location. Notable additional infections detected included a novel mycosis and two new Haplosporidian species, which again, were only found in the Pier location.

Due to the increasingly wide range of C. *maenas*, as well as the site-specific differences seen, it is imperative to further understand the ecology of these diseases in terms of environment and connectivity alongside traditional diagnostics.

MICROBIAL CONTROL DIVISION SYMPOSIUM - PROMISING MICROBIAL CONTROL OPTIONS FOR FALL ARMYWORM, A GLOBAL

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I am a biopesticide specialist working for over 35 years initially as a research scientist then for biocontrol companies and now as an independent specialist. I gained an MSc in Technology for Crop Protection and a PhD in Biological Control both from Reading University. I have taken a lead role in the development and registration of many new biocontrol technologies. My expertise is in facilitating the process of getting biocontrol products onto the market by streamlining bio-discovery, product development, registration and marketing.
I have been an expert for regulation of biocontrol agents (OECD, EU, FAO/WHO). I am currently Vice-President of IBMA. I was editor of the BCPC 'The Manual of Biocontrol Agents' and support CABI for their Bioprotection portal.

An a priori strategy for using market-ready microbial biocontrol products for FAW control: technical, economic and end-user consideration

Fall Armyworm (FAW, *Spodoptera frugiperda*) has spread globally and tonnes of maize could be lost annually due to FAW. Some chemical pesticides being used against FAW are toxic to humans and cause environmental contamination therefore there is an urgent need to find biocontrol products which minimize harm and to use them in Integrated Pest Management (IPM) which takes a 'biocontrol first' approach. But, as FAW is a new pest, there are few biocontrol products available. Therefore, what is the best way to quickly find suitable FAW products? A research programme of strain discovery and screening takes 5-10 years while for a commercial development pathway this timeline is reduced, it still takes 4-6 years. The cost of developing a new product can easily be at least $30 million. Therefore, the best way to move quickly is to identify existing biocontrol products with activity against FAW, such as products already available for related Spodoptera spp. This means that products are already commercially available with a registration dossier and within 1-2 years these products can available to farmers. These products may not be the most effective strains against FAW but to move fast they can be offered to farmers while the longer research of finding the best strain takes place.

In conclusion, farmers need to have rapid access to suitable FAW-active biocontrol products and be trained in 'biology first' IPM. This approach includes facilitating the regulatory process and reducing barriers.

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Rica Joy Flor works with the International Rice Research Institute as a Scientist on Innovation Systems, based in Phnom Penh, Cambodia. She has a background in Anthropology from the University of the Philippines and a PhD in technology and innovation studies from Wageningen University, Netherlands. Her research interests are on drivers and constraints to adoption of technologies. She also investigates how innovations targeting sustainable crop production can spread widely. In these, she studies the underlying social structures and interaction patterns that affect transitions to more sustainable agricultural systems.

Working with multiple stakeholders on *Beauveria bassiana*: Testing a commercially available biocontrol for fall armyworm in Cambodia

Cambodian farmers have options for pesticide based pest management, but limited access to biocontrol. One commercially available product is *Beauveria bassiana*, but there is little awareness and adoption. Research since 2017, was done to test and locally adapt *B. bassiana* as part of rice IPM. In 2019-2020, experiments were done to compare *B. bassiana* and commercially available pesticides against fall armyworm (FAW, *Spodoptera frugiperda*) on maize. This study presents the process of technological adaptation through farmer-participatory experiments, as well as the adoption outcomes from surveys of farmers in 2016 and 2019 (N=199, paired data). We also summarize the results of the experiment on FAW from two cropping seasons.

Farmers made technological adaptations to integrate use of *B. bassiana*. Private sector stakeholders also tested institutional innovations to enable adoption. We found limited adoption of *B. bassiana* (2%) after four years, but farmers significantly reduced their insecticide application (P=.004).

On FAW, the treatment with *B. bassiana* consistently showed higher damage compared with two pesticide treatments, across two seasons. However, it had significantly lower damage compared with control (do nothing). Compared with pesticide treatments, the *B. bassiana* treatment had higher yield in one season, and lower yield in the other. Based on these results, we reflect on what is needed to further the adoption of *B. bassiana* as management option for FAW in Cambodia.

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Dr Sevgan Subramanian, Principal Scientist and Head of Environmental Health Theme, icipe has over 17 years of Insect Research for Development (R4D) experience in Asia and Africa. He holds holds a PhD and MSc in Agricultural Entomology from Tamil Nadu Agricultural University, India. His R4D focus are in the areas of integrated pest management (IPM) strategies for staple and horticultural crops, Biopesticides, climate change adaptation, and Insects for food and feed. He has more than 150 publications, with over 109 peer-reviewed articles in high impact journals. Dr Subramanian has contributed extensively to building African research capacity in IPM, climate change and edible insects and guided 4 Postdoc, 12 PhD and 8 MSc scholars.

Development of Biopesticides for sustainable management of Fall armyworm in East Africa

Maize contributes to the food security of >300 million people in sub-Saharan Africa. The invasion of Africa and subsequent damage by fall armyworm (FAW), *Spodoptera frugiperda* has emerged as a threat to food security. Wide use of pesticide against FAW are costly, often ineffective, and detriment to environment. Natural epizootics of entomopathogenic fungi (EPF) and baculoviruses widely reported on various life stages of FAW highlight their potential for use in FAW management. Rapid screening of strains from the entomopathogen repository in icipe has resulted in the identification of potent isolates of *Metarhizium anisopliae* (ICIPE 7, 78 and 41) against egg and neonates and *M. anisopliae* (ICIPE 30, 7) and *Beauveria bassiana* (ICIPE 621) against adults of fall armyworm. Through effective Public-private partnerships, field efficacy of isolates ICIPE 7 and 78 have been demonstrated and are being currently progressed as biopesticide products for FAW management. Compatibility of EPFs with FAW pheromones and 80 – 85% decline in fecundity and more than 90% decline in egg viability with EPF infection highlight the potential for innovative "lure and infect" strategies. Further research on exploiting endophytic property of EPFs, integration with diversified maize cropping systems, natural enemies, better timing of application and small-scale production of EPFs needs to be strengthened for development of biopesticides as a sustainable management option for fall armyworm in Africa.

Prof. Italo Delalibera Júnior

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Professor at the University of São Paulo (ESALQ-USP) with a Ph.D. in Entomology from Cornell University (2002) and post-doctorate from the University of Wisconsin (2003). He has experience in Agricultural Entomology, and his research focuses on Microbial control of insects and mites with entomopathogenic fungi. He is the director of the EMBRAPII unit (Brazilian Company for Industrial Research and Innovation) accredited to work with Biocontrollers and biotechnological processes in the sustainable management of agricultural pests.

Progress in Brazil for the control of FAW with microbial pathogens.

Spodoptera frugiperda (FAW) is an important pest of the main crops in Brazil, such as corn, cotton, soybeans, rice, and sorghum, and it can also feed on several cultivated and non-cultivated plants. The standard control measures such as chemical pesticides and transgenic plants are becoming ineffective due to cases of resistance. The use of biological control in the country is increasing rapidly. There are 11 commercial products based on Bacillus thuringiensis and 5 Baculovirus products currently available in the country. *Baculovirus spodoptera* and *Bacillus thuringiensis* have excellent efficacy for controlling small caterpillars (up to the third instar, <8mm). For these reasons, the timing of application is crucial. It is essential to apply right after the first signs of scraped leaves and apply it at dusk due to the caterpillar's nocturnal habit and to reduce inactivation by UV radiation. Entomopathogenic fungi can infect larger caterpillars and have great potential against FAW, but there is no commercial product in the market. Epizootics of the fungus *Metarizium rileyi* naturally reduce FAW populations drastically. However, *M. rileyi* is more fastidious, and it seems unstable for continuous production by solid substrate fermentation. Other fungi such as *Beauveria bassiana* e *Cordyceps fumosorosea* can also be effective, and their production is well established. They can also colonize plants endophytically and induce plant defence against FAW. Maize plants inoculated with *Metarhizium* spp., for example, resulted in low survival of FAW, revealing the potential of a new control strategy.

VIRUS DIVISION DYMPOSIUM : PLACE OF BACULOVIRUSES IN THE FIGHT AGAINST COVID-19

Dr Linda King

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Linda King completed her D. Phil. in insect virology at the University of Oxford in 1985. Linda then undertook a post-doc position in the Department of Biochemistry, and a Research Fellowship at Linacre College, Oxford working on human papilloma viruses. She then moved to a lectureship at Oxford Brookes University where she established the Insect Virus Research Group. Following promotion to Professor of Virology and spells as Head of Department of Biosciences and Associate Dean for Research in the Faculty of Health & Life Sciences, Linda is currently Pro Vice Chancellor for Research & Global Partnerships. In 2007, she co-founded Oxford Expression Technologies Ltd to help commercialise the *flash*BAC baculovirus expression system.

Four decades of the Baculovirus Expression System – from early beginnings to being at the forefront of global efforts against COVID-19

Baculovirus expression vectors have become established as some of the most widely used protein production systems for both academic and commercial applications. This talk will explore the origins of the system, which was first presented at an SIP meeting in Brighton in 1983, and its evolution over the last four decades. Basic knowledge and understanding of the virus and its interactions with the host insect cell have enabled the expression system to be improved both in terms of ease of use by non-experts, and in terms of versatility to produce large quantities of high quality proteins. Both of these aspects have contributed to the popularity of the expression system across the world. Pioneering work within companies has demonstrated the utility of the system to produce safe and effective vaccines. And thus it is perhaps not surprising that the baculovirus-insect cell expression system is at the forefront of global efforts against COVID-19 – not just in the development of a safe and effective vaccine but also in the production of proteins to aid research into SARS-CoV-2 and its immune responses.

Dr. Gorden Pijlman

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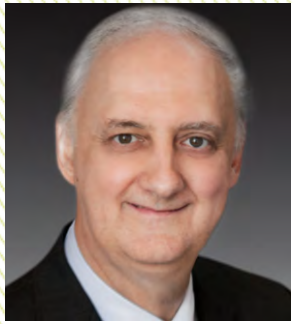
Gorben Pijlman obtained his PhD in the baculovirus research group of prof. Dr. Just M. Vlak. In 2003 he joined the Flavivirus research group of prof. Alex Khromykh at the University of Queensland in Brisbane, Australia, for postdoctoral research on West Nile virus. In 2007, he returned to Wageningen and now has a permanent position as Associate Professor in Arbovirology. The current research programme is an interesting mix of fundamental virology focused at arbovirus-host interactions and applied studies on arbovirus vaccine development. Since early 2013 the arbovirus group conducts arbovirus transmission studies using live mosquitoes and class pathogenic arboviruses (Chikungunya, West Nile, Usutu and, since 2016, Zika virus), in a purposely-built biosafety level 3 (BSL3) laboratory at Wageningen campus. At present, the group works on a COVID-19 vaccine from insect cells (H2020 Prevent-nCoV consortium).

Two-component nanoparticle vaccine displaying glycosylated spike S1 domain induces neutralizing antibody response against SARS-CoV-2 variants

Vaccines pave the way out of the SARS-CoV-2 pandemic. Besides mRNA and adenoviral vector vaccines, there is a need for effective protein-based vaccines for immunization against current and emerging variants. We have developed a virus-like particle (VLP)-based vaccine using the baculovirus-insect cell expression system, a robust production platform known for its scalability, low cost, and safety. Baculoviruses were constructed encoding SARS-CoV-2 spikes: full-length S, stabilized secreted S, or the S1 domain. The antigens were found reactive to sera of COVID-19 convalescent patients. Since subunit S only partially protected mice from SARS-CoV-2 challenge, we produced S1 for conjugation to bacteriophage AP205 VLP nanoparticles using tag/cather technology. The S1 yield in an insect-cell bioreactor was ~11 mg/Liter and authentic protein folding, efficient glycosylation, partial trimerization and ACE2 receptor binding was confirmed. Prime-boost immunization of mice with 0.5 microgram S1-VLPs showed potent neutralizing antibody responses against Wuhan and UK/B.1.1.7 SARS-CoV-2 variants. This two-component nanoparticle vaccine can now be further developed to help alleviate the burden of COVID-19.



Dr. Gale Smith

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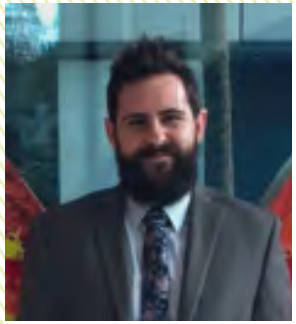
Dr. Gale Smith, PhD is the Senior Vice President of Vaccine Development and Chief Scientist at Novavax, Inc, Gaithersburg, MD. Dr. Smith leads a team of scientists responsible for design and scientific direction of programs on vaccine development against pre-pandemic influenza, seasonal influenza, COVID-19, SARS, MERS, Ebola, COVID-19 and other infectious diseases. Currently, Dr. Smith leads the scientific team that developed a SARS-CoV-2 spike nanoparticle COVID-19 vaccine proven to be well tolerated and highly protective in phase 3 human trials in the UK, South Africa, and US. Dr. Smith received a PhD in Microbiology from Texas A&M University where he co-invented the BEVS baculovirus insect cell expression vector system and holds numbers patents including for the first recombinant influenza vaccine Flublok™.

Baculovirus-Sf9 Insect Cell Technology in the Development of a COVID-19 Vaccine

The baculovirus expression vector system (BEVS) is a proven technology for the development and production of vaccines including a leading subunit vaccine to address the urgent global need to prevent disease and spread of COVID-19. Discussed will be strengths of BEVS for the development of NVX-CoV2373 vaccine, a protein-based vaccine engineered from the genetic sequence of SARS-CoV-2 spike glycoprotein. NVX-CoV2373 recombinant spike (rS) created from the coronavirus SARS-CoV-2 spike protein is full length, stabilized, and in a properly folded prefusion conformation. Presented will be unique properties of rS made in Sf9 insect cells that contribute to dynamic structure, proper folding, and broad epitope presentation along with high resolution examples and how each may contribute to protection. NVX-CoV2373 vaccine in a 30,000 person pivotal Phase 3 trial in the US demonstrated efficacy of 100% against the moderate and severe disease and 93.2% against emerging variants.

FUNGI DIVISION SYMPOSIUM : NEW ADVANCES IN THE WORLD OF THE ENTOMOPHTHORALES



Dr Brian Lovett

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Brian Lovett is a postdoctoral researcher at the Division of Plant and Soil Sciences at West Virginia University working on entomopathogenic fungi and biotechnology. He has contributed to the advancement of transgenic mosquito-killing fungi for malaria prevention.

The patient puppetmaster: how *Massospora* spp. infect and manipulate cicada hosts

Fungi from the genus *Massospora* are specialized cicada parasites. These species enjoy worldwide distribution, and each is typically limited to a single genus of cicadas. Annual and periodical cicadas are known to fall victim to different species. The etiology of this interaction follows a stereotyped progression that begins when the cicada encounters *Massospora* spp. resting spores in the soil. These unlucky cicadas develop a conidial infection that involves replacement of the posterior end of the abdomen with a chalky, yellow plug of fungus. This "mass of spores" is spread via active host transmission; ultimately, the host cicada is chemically enlisted by the fungus to infect other individuals. At this stage, infected annual and periodical cicadas were found to contain psychoactive metabolites. The subsequent infections develop into resting spore phase, which release spores into the soil that patiently remain until the next cicada emergence. Despite their first description in 1888, much about the biology of this bizarre parasite remains obscure. In this talk, I will describe historical and modern investigations of *Massospora* spp. and place them in context with other entomophthoralean fungi. The lifestyle of this fungus provides perspective on the spectrum that parasites and pathogens lie upon and how host specialization can result in host manipulation. Additionally, I will discuss how the cicada phenology may benefit the pathogen, but presents a challenge to scientific inquiry.

Dr Carolyn Elya

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Carolyn completed her B.A. in Biochemistry and Molecular Biology from Reed College in 2009, then went on to join Michael Eisen's lab as a graduate student in the department of Molecular and Cell Biology at University of California, Berkeley. During her graduate work, Carolyn discovered, isolated and began studying a strain of the entomopathogenic fungus Entomophthora muscae that naturally infects fruit flies. After completing her Ph.D. in 2017, Carolyn joined the lab of Benjamin de Bivort in the department of Organismic and Evolutionary Biology at Harvard University and became an HHMI Hanna Gray Fellow. She is working to understand the neurobiological and molecular underpinnings of "zombie" behaviors elicited by *E. muscae*.

Taking control: Mechanistic insights into the behavioral hijacking of fruit flies by the zombie fungus *Entomophthora muscae*

Some fungal pathogens elicit striking behavioral changes in their insect hosts, but the underlying molecular and neurobiological mechanisms are poorly understood. This is due in part to the difficulty of studying non-model organisms with limited tools. Recently, I discovered a strain of the Entomophthora muscae in wild Drosophila and developed methods to propagate the fungus in the model organism D. melanogaster. Before sunset on their final day of life, infected flies manifest the moribund behaviors characteristic of E. muscae infections: they climb to a high location (a phenomenon known as "summiting"), extend their proboscises, and raise their wings in a pose that facilitates spore dispersal. Currently, we are using the E. muscae-fruit fly system to determine the mechanistic underpinnings of summiting behavior. We have developed an automated behavioral assay to measure summiting and now understand summiting to consist of a burst of locomotion beginning approximately two hours before death. Using this assay as the basis of candidate screen, we have identified a putative summiting circuit consisting of clock neurons, neurosecretory neurons and the corpora allata. We hypothesize that the fungus modifies this circuit by secreting neuromodulatory metabolites into the fly open circulatory system, a mechanism consistent with metabolomics profiling of hemolymph of summiting flies and enhanced permeability of the nervous systems of infected flies. We will next use functional imaging to assay the impact of hemolymph from summiting flies on neural activity. With these and other efforts, we aim to understand the mechanistic basis of summiting, from fungus to fly.

Dr Andreas Naundrup Hansen

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I am a biologist with a great interest in pathogen-induced host manipulation and chemical ecology.

I received my master's degree in Molecular Biology and Genetics in 2017 and recently received a PhD degree in June 2021, both from the University of Copenhagen. During my PhD, which I conducted under the supervision of Henrik H. De Fine Licht and Annette Bruun Jensen, I worked on the interactions between the insect pathogenic fungus, *Entomophthora muscae* and the housefly (*Musca domestica*) host.

A pathogenic fungus uses volatiles to entice male flies into fatal mating attempts with infected female cadavers

The obligate pathogenic fungus *Entomophthora muscae* infects and kills houseflies (*Musca domestica*). Prior to host death and fungal sporulation, the fungus manipulates its host to seek an elevated position and take a characteristic body stance, a behavior called summiting. We investigated the housefly attraction to sporulating, deadly cadavers using a combination of chemical analysis, behavioral experiments and transcriptomics. Gas Chromatography-Mass spectrometry revealed large changes in cuticular and headspace profile, including upregulated housefly pheromones and novel compounds only found in sporulating flies. Behavioral experiments showed an increase in male sexual activity towards sporulating female cadavers, attraction of both males and females to infectious conidia, and attraction to headspace extracts of sporulating flies in a two choice experiment. When the headspace extract was analyzed in Gas Chromatography coupled to Electroantennal detection, several compounds only found in sporulating fly headspace elicited an antennal response. Lastly, transcriptomics of fungus- and fly genes revealed several genes upregulated related to the biosynthesis of biologically active volatiles found sporulating headspace. Collectively, these findings indicate the first instance of an adaptive manipulation strategy where the fungus not only manipulates the focally infected host to induce summiting, but also to actively attract novel hosts for infection after host death.

Dr Linda Muskat

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Linda received her MSc in Biology from Mainz University in 2016. Her interest in fungi was spawned during her Master thesis, dealing with fungal antagonists and their secondary metabolites as potential biocontrol agents against plant pathogenic fungi. In 2017 she joined in the Patel lab at the Bielefeld University of Applied Sciences and is currently completing her PhD in cooperation with the Agricultural Entomology at Göttingen University. Her current research focusses on the development of innovative formulations to improve delivery of entomopathogenic fungi for psyllid pest control. Linda´s further research interests are computerized quantification of fungal spores, formulation materials for stimuli-triggered release of semiochemicals and artificial insect diets.

Fermentation and formulation of *Pandora* sp. nov. for biological psyllid pest control

Despite their potential for pest control, no biological control agents based on entomphthoralean fungi have been commercialized so far. This can be attributed to the difficulties with cultivation and formulation of these fungi and the variation in spore production over time. The project PICTA-KILL aims to explore the potential of a newly discovered *Pandora* species for biological psyllid pest control in fruit orchards. First, a cost-effective and scalable fermentation process for finely-dispersed hyphae was established to cultivate the new fungal isolate. Subsequent encapsulation in a hydrogel matrix supported sporulation. In laboratory infection experiments, the target insects *Cacopsylla picta* and *C. pyri* were successfully killed by the fungus sporulating from capsules. Moreover, to accelerate the quantification of conidial discharge from capsules, a computer-assisted image analysis method was developed. Besides, a new superabsorber formulation increased sporulation at reduced humidities under field conditions. To increase the chance of contact between target insect and fungus, we further developed a novel formulation for the temperature-triggered release of *C. picta* attracting semiochemical ß-caryophyllene. Our studies form the scientific-technical foundation for above-ground application of entomophthoralean fungi against psyllid pests.

Dr Stéphanie Saussure

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My focus lies in the understanding and use of ecosystem services for sustainable food production. By combining agroecology, insect pathology and modelling, I investigate (i) pest-natural enemy interactions, and (ii) the effect of agricultural practices and local landscape on pests, beneficial organisms, and biodiversity. I am convinced that a multidisciplinary approach is necessary to adapt plant protection to a changing environment and to efficiently implement potential new strategies for Integrated Pest Management.

I did my PhD in Norway at NIBIO and NMBU on the interactions between aphid pests and their fungal natural enemies in cereals. Currently, I am working at Luke, Finland, as a Postdoc and study the influence of different agroecological practices on biocontrol and pollination.

Can fungal epizootics reduce yield loss caused by aphids in cereals?

Sitobion avenae is a common European aphid pest in cereals. *Pandora neoaphidis* is among its most prevalent fungal disease. Under favorable conditions, *P. neoaphidis* may cause epizootics that result in the host population crash and, potentially, reduce yield loss. Understanding the mechanisms and necessary conditions to maximize biocontrol and reduce yield loss is important for Integrated Pest Management.

We built a mechanistic tri-trophic model between winter wheat, *S. avenae* and *P. neoaphidis*, that simulates the aphid-fungus dynamics and the associated yield loss from spring to harvest. Aphid immigration was fixed to a level enabling an outbreak in the fungus absence. Further, the fungus inoculum was modelled as a proportion of infected immigrants. Each simulation was run twice, with and without *P. neoaphidis*, to estimate the biocontrol. Uncertainty in model parameters and variation in weather were included, resulting in a range of simulation outcomes. A global sensitivity analysis identified the most important parameters influencing the yield loss.

In our model, not all epizootics maximized the biocontrol. The fungus transmission efficiency, the humidity threshold triggering fungal sporulation and the weather (temperature and relative humidity) were crucial to explain yield loss. The fungus inoculum quantity was only moderately important. Finally, the cadaver prevalence at the beginning of flowering and milk stage gave an indication of the final biocontrol level.

Dr Ann E.Hajek

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Dr. Ann Hajek is a Professor of Invertebrate Pathology in the Department of Entomology at Cornell University, Ithaca, New York. She has taught invertebrate pathology courses at Cornell and in recent years has conducted insect pathology short courses. The emphasis for her research has been in epizootiology and evolution of entomopathogenic fungi and she has also worked with nematodes and microsporidia. Dr. Hajek has been a member of the Society for Invertebrate Pathology for more than 30 years. She has undertaken many leadership roles in the society, including Secretary and Treasurer of the society andshe has helped to organize several meetings.

Batkoa major infecting spotted lanternflies: Host range and population structure

The entomophthoralean genus *Batkoa* was named in 1989. The eleven species presently in the genus had previously been placed in numerous different genera, including *Conidiobolus*, *Entomophaga*, and *Entomophthora*. The species *Batkoa major* was last reported from North America in 1888, infecting a small beetle in the southeast. Therefore, it was surprising to find epizootics caused in part by *B. major* in populations of the new invasive fulgorid, the spotted lanternfly, *Lycorma delicatula*, in the northeastern United States in 2018. We asked what native hosts are infected by *B. major* in this region and found infections in native insects in 5 different insect orders: Diptera, Hemiptera, Coleoptera, Lepidoptera, and Psocoptera throughout the season. Among 62 of the samples with the most accurate host identifications, most (34) were from Diptera, but the next most common hosts were hemipterans (11), including several planthopper families (although not Fulgoridae as this region hosts no native species of fulgorids). Analyses of population structure demonstrated limited variability within *B. major* that appeared to differ by geography. We will discuss the fact that the broad host range of *B. major* is not consistent with findings of narrow host ranges for many entomophthoralean species.

MICROSPORIDIA DIVSION SYMPOSIUM : MICROSPORIDIA OF INVERTEBRATE HOSTS IN AQUATIC AND TERRESTRIAL HABITATS

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Dr. Aaron W. Reinke is an assistant professor in the Department of Molecular Genetics at the University of Toronto. Dr. Reinke did his graduate training at the Massachusetts Institute of Technology with Prof. Amy Keating, using biochemical approaches to determine how proteins correctly choose their interaction partners. As a Life Science Research fellow at the University of California, San Diego in the lab of Prof. Emily Troemel, Dr. Reinke studied parasite infections, using a unique and powerful system of a tractable host, C. elegans, and a coevolved intracellular eukaryotic parasite, N. parisii. Dr. Reinke started his lab in the fall of 2017 has received several young investigator awards including a 2019 Sloan Research Fellowship in Computational & Evolutionary Molecular Biology.

Generation of a database of microsporidia species characteristics

Microsporidia are a large group of fungal-related obligate intracellular parasites. Though many microsporidia species have been identified over the past 160 years, there is a lacking depiction of the full diversity of this phylum. To systematically describe the characteristics of these parasites, we created a database of 1,440 species and their attributes, including the hosts they infect and spore characteristics. We find that microsporidia have been reported to infect 16 metazoan and four protozoan phyla, with smaller phyla being underrepresented. Most species are only reported to infect a single host, but those that are generalists are also more likely to infect a broader set of host tissues. Strikingly, polar tubes are 3-fold longer in species that infect tissues besides the intestine, suggesting that polar tube length is a determinant of tissue specificity. Phylogenetic analysis revealed four clades which each contain microsporidia that infect hosts from all major habitats. Although related species are more likely to infect similar hosts, we observe examples of changes in host specificity and convergent evolution. Taken together, our results show that microsporidia display vast diversity in their morphology and the hosts they infect, illustrating the flexibility of these parasites to evolve new traits.

Dr Yuliya Sokolova

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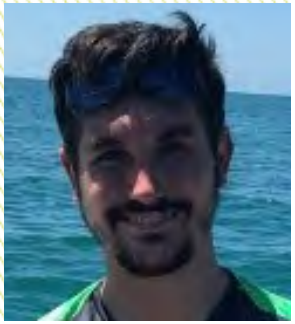
I graduated from St.Petersburg (SPb) State University, Russia. My affair with microsporidia started in 1984 when I joined PhD program with Irma Issi's lab, All-Russian Institute for Plant Protection (VIZR). After obtaining PhD in 1990, I worked as a researcher in VIZR, and later in the Institute of Cytology, SPb. Since 2000 I have been working primarily in the USA: first in Jim Fuxa's lab in Louisiana State University, Baton Rouge, later in Liz Didier lab in Tulane University, New Orleans, LA, and then again in Baton Rouge running EM core with LSU SVM. I got Dr. Sci. degree in 2018 in the Institute of Zoology, SPb (dissertation "Cell biology and biodiversity of Microsporidia"). Since then my research shifted more towards cell biology and advanced EM methodology. I am working with NIH now.

Microsporidia in trematodes: an overview and new findings in the USA and Russia

Due to lifecycles involving multiple hosts of various trophic levels and taxonomic groups, trematodes provide opportunities for their hyperparasitic microsporidia (M) to hitchhike between invertebrate and vertebrate hosts representing thus a potential route for transmission and radiation. Up today, 36 species of M were recovered from digenean hosts, twice that reported by Sprague (1977). Nine of those belong to the genus *Unikaryon* that includes specialized parasites of Platyhelminthes, 4 - to two closely-related genera of fish microsporidia, *Pleistophora* and *Ovipleistophora*, and remaining 23 species that we place in the *incertae sedis* group, are assigned to *Nosema* (14 species) and Microsporidium (8 species). Two species of the *Pleistophora-Ovipleistophora* group and all Unikaryon spp. were studied by electron microscopy. SSUrDNA sequences of only three species (*U.legeri, U.panopei and O.diplostomuri*) are available from GenBank. It is likely that M infections of digeneans are not uncommon, and that many new species remain to be discovered. Our recent pilot surveys for trematode-infecting M in Florida, USA, and St. Petersburg, Russia, confirm this assumption: two new Unikaryon species were recovered from microphallid metacercariae parasitizing Florida intertidal crabs, and at least two species (their characterization is in progress) – from rediae and sporocysts of echinostomatids and diplostomatids infecting 6 species of gastropods in St. Petersburg freshwater lakes and ponds.



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My research involves screening for and systematically identifying the symbionts of invertebrate hosts; primarily including Crustacea and Mollusca. I work mainly with Microsporidia, bacteria and viruses, and use histological, TEM, molecular diagnostic and metagenomic methods to better understand the microbiomes of invertebrate hosts. Follow me on Twitter: @JamieBojko

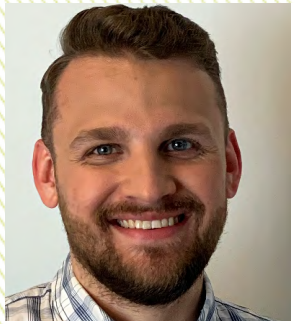
A new microsporidian parasitizing invasive Carcinus sp. in the Argentinian Atlantic

Co-authors : Jamie Bojko, Antonella Frizzera, Nuria Vazquez, Florencia Cremonte

Microsporidians associated with aquatic environments total ~70% of known microsporidian diversity. Up to 23% of these parasites have been identified marine environments, most predominantly in crustacean hosts. As well as hosts to a range of microsporidian parasites, the Crustacea are also common biological invaders of aquatic habitats. To date, >320 crustaceans are invasive or non-native, globally. Some, such as the invasive green crab, *Carcinus maenas*, have entered environments across the USA, south America, Asia, Australia, and Africa, constituting a world-wide risk.

Carcinus maenas was recently identified from marine environments surrounding Argentina. This species has been associated with over 80 different symbionts, some that pose a risk to native fauna. Our study, which is funded by the Global Challenge Research Fund (GCRF) and Conicet-Cenpat (CCT), explored the pathogen profile of >100 *Carcinus* sp. to determine whether any pathogenic organisms had been carried into the naïve marine environment. Our screen used histopathology as a primary data source, followed by metagenomic analyses of crab tissues.

Our findings include a novel microsporidian pathogen of the connective tissues and reserve inclusion cells of a Carcinus sp. host. Phylogenetic analysis of the microsporidian relates it to *Agmasoma penaei* (82% similarity), a Clade IV microsporidian of penaeid shrimp. The site of infection and genetic data suggest that this microsporidian belongs to a new genus, pending developmental and ultrastructural data. Given the pathogenic data derived from *A. penaei* and its impact on aquaculture, we consider the impact that may be present within Argentinian fisheries and local ecosystems.



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Artur Trzebny has BSc in Biology and Master of Science in Biotechnology from the Adam Mickiewicz University in Poznan, Poland (AMU). Since 2017, he is a PhD student at Faculty of Biology, AMU, and his thesis concerns on biodiversity and ecology of Microsporidia. He has developed new methods for the detection of microsporidian species and their hosts based on a DNA-metabarcoding approach. Recently, he started research on host microbiome changes caused by microsporidian infection, including other organisms vectored by or pathogenic to the host. Since 2019 he is the Student Representative Officer at SIP Microsporidia Division. In spare time, when he's not catching microsporidia-infected mosquitoes, he loves cycling, swimming, and horse riding.

Specific mosquito gut microbiome members are associated with microsporidian infection

Insect gut microbiota mediate the interactions between a host and intestinal parasites by stimulating host immune responses or protecting the host by inhibiting parasites. However, only sparse studies concern parasite effects on host's gut microbiome structure and changes in microbial metabolic pathways. Our study is a first attempt to resolve this question using microsporidian infections in natural mosquito populations as a model.

In total, 188 field-collected adult mosquito females were analysed in which 108 were infected by 11 microsporidian species. Microbial taxa were detected in each mosquito by sequencing of the V4 region of 16S rRNA gene. We found higher abundance of Spiroplasma sp. PL03 in microsporidia-positive mosquitoes, while *Weisella* cf. viridescens was observed only in infected ones. Moreover, during microsporidian infection, we observed in bacteria an increase in biosynthesis of some antibiotics and the pathways involved in oxidative phosphorylation and pentose synthesis.

Our results support hypotheses that microsporidians might manipulate host's metabolism and biological processes of gut endosymbiotic bacteria to promote nucleotide synthesis and thus increase the amount of ATP and nucleotides that can be imported by microsporidian-infected cells.

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Prof. Daniela Pilarska

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Dr. Daniela Pilarska is a professor at the Department of the Natural Sciences at New Bulgarian University and at the Institute of Biodiversity and Ecosystem Research, the Bulgarian Academy of Sciences. She earned a Ph.D. in Parsitology at the Department of Parasitology, Faculty of Natural Sciences, Charles University, Czech Republic where she studied with Professors Jaroslav Weiser and Jiri Vavra. Her research has focused on microsporidian pathogens of pest insects, primarily gypsy moth, Lymantria dispar, and browntail moth, Euproctis chrysorrhoea. In 2001-2002 she was awarded a Fulbright fellowship and conducted research at the laboratory of Dr. Leellen Solter at the Illinois Natural History Survey. Her current research focuses on microsporidia, entomopathogenic fungi and biological control of pest insects.

A review of research on microsporidia infecting pest insects in Bulgaria

Microsporidia are obligate, intracellular pathogens infecting all major animal taxa. Some species play an important role as natural regulatory agents of host insect populations. Investigations of insect microsporidia in Bulgaria began in 1960, however systematic research on microsporidia was initiated in the 1990s in close cooperation with scientists from Germany, Czech Republic, Austria, Slovakia and the USA. Since then, 27 microsporidian species in the genera *Nosema, Vairimorpha, Endoreticulatus, Cysposporogenes, Amblyospora, Janacekia, Polydispyrenia, Thelohania, Bohuslavia* and *Chytridiopsis* have been recovered from pest insects in the orders Orthoptera, Coleoptera, Diptera and Lepidoptera. Special attention was paid to microsporidia parasitizing gypsy moth, *Lymantria dispar*, and brown tail moth, Euproctis chrysorrhoea, both defoliators of multiple tree species. Three new species, *Vairimorpha disparis, Nosema chrysorrhoea* and *Endoreticulatus poecilimonae* were described from the lepidopterans L. *dispar*, E. *chrysorrhoea* and the grasshopper *Poecilimon thoracicus*, respectively. Here, the diversity of microsporidia in Bulgarian insects and data about their biology and phylogeny are presented.

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Yuri Tokarev has started his career in Microsporidia research in 1998 under supervision of Professor Irma V. Issi at the All-Russian Institute of Plant Protection, St. Petersburg, Russia. After graduation from St. Petersburg State Agrarian University in 1999 he has continued education at the St. Petersburg State University and defended his Master thesis in 2001, then PhD thesis in 2003 and Doctoral thesis in 2013. The main research area is Microsporidia diversity, taxonomy, molecular phylogeny and parasite-host interactions, as well as implications for microbial pest control. The current position is leading researcher of Laboratory of Molecular Plant Protection. Professor of Russian Academy of Sciences and Deputy Editor of the journal "Plant Protection News" (http://plantprotect.ru).

How do microsporidia of insect hosts interact with insect parasitoids?

Microsporidia are widespread in insects, notably contributing to density dynamics of Lepidoptera, and implications for pest microbial control have been considered. However, switching of microsporidia between insect hosts from different orders is not uncommon, emphasized by ecological links between the latter. This means that microsporidia in lepidopteran insects may affect insect parasitoids. What are the consequences of this co-occurrence of the parasites? From the body of published works, it can be concluded that microsporidia, primary insect hosts and insect parasitoids establish complex interactions with different scenarios, depending upon the species and conditions under which they interact. On one hand, both microsporidia and parasitoids suppress insect host immune system, making the host organism more favorable for development of each other. On the other hand, microsporidia and parasitoids compete for the host resources, adversely affecting development of each other. Microsporidia with a broad host range are capable of infecting the parasitoids, sometimes causing more detrimental diseases as compared to the primary insect host infections. For example, when reared on *Ostrinia nubilalis* infected with *Nosema pyrausta*, larvae of *Habrobracon hebetor* succeeded to pupation but adults failed to emerge, and the perished pupae were packed with microsporidia spores, which seemed to be the reason for 100% premature mortality. *Cotesia glomerata* could not establish in Pieris brassicae larvae infected with *Nosema tyriae* and *Nosema* cf *polyvora* (as opposed to microsporidia-free larvae used as control). We speculate that early premature stages of the parasitoid were killed by microsporidia. Supported by RSF, project # 20-66-46009.

DISEASES OF BENEFICIAL INVERTEBRATES & VIRUS CROSS-DIVISION SYMPOSIUM : VIRUS OF POLLINATORS

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Adam is an Assistant Professor in the Department of Entomology and the University of Illinois Urbana-Champaign. He teaches courses in Physiology (IB 202) and Genes and Behaviour (IB 432) in the School of Integrative Biology. He studies how pollinators, mostly focusing on honey bees, respond to the different stresses they encounter in their environment. These studies have centred heavily around the impacts of virus infection and the nutritional and chemical stresses associated with Midwestern row-crop agricultural systems. Adam received his Ph.D. from Arizona State University, where he studied the behavioural physiology of harvester ants and honey bees; he then did postdoctoral work at Iowa State University.

Effects of Honey bee virus causes context-dependent changes in host social behaviour

Social insect colonies often consist of dense populations of closely-related individuals, presenting advantages to pathogens. Further, many social insects, including honey bees, have reduced physiological immune responses, instead relying more on behavioural immune responses. These adaptations can reduce transmission of diseases both within and between colonies. However, anthropogenic changes can create evolutionarily novel environments that present opportunities for emerging diseases, potentially changing the balance of host-pathogen co-evolution. Recent intensification and globalization of honey bee management has coincided with increased pathogen pressure, increasing the likelihood for novel pathogens to exploit managed environments. Using a mixture of manual and automated behavioural monitoring, we investigated how treatment with Israeli acute paralysis virus (IAPV) or a non-infectious dsRNA immunostimulant affected honey bee behaviour and physiology. We found that both treatments elicited seemingly adaptive behavioural responses, specifically behavioural changes likely to reduce pathogen transmission within a colony. However, IAPV infection causes pathogen-specific changes in behaviours related to intercolony transmission, suggesting that IAPV can manipulate host behaviour to enhance free movement to susceptible colonies. These effects are most likely to be beneficial in the unnaturally high colony densities produced by modern apicultural practices.

Dr Eugene Ryabov

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Eugene Ryabov, is an invertebrate and plant virologist. A graduate from Moscow State University, he initially worked on the mechanisms of viral movement within plants and on plant antiviral RNA defences. Later, he extended his research to include the viruses of aphids and honey bees. His current work on honey bee viruses, initiated in the in UK and currently being continued at the USDA Beltsville Bee Research Lab in the USA, focuses on the impact virus infections on honey bee health, interactions between honey bee viruses and their mite vector *Varroa destructor*, viral genetic diversity and evolution, and the mechanisms of antiviral responses. His research includes the development and the use of infectious cDNA clones of the viruses, including those of Deformed wing virus.

Reverse genetic system for Deformed wing virus, a principal honey bee pathogen.

Deformed wing virus (DWV), a positive strand RNA virus, is the major honey bee pathogen associated with colony losses. DWV became highly virulent with the spread of its vector, an ectoparasitic Varroa mite. Studies of the interactions between DWV, *Varroa*, and honey bees are complicated due to the virus presence at low levels even in *Varroa-free* colonies, high genetic diversity, and multiple transmission routes. We developed a series of full-length infectious cDNA clones for several isolates of DWV, as well as a system for cloned virus recovery. The ability to precisely trace genetic changes in the clone-derived progeny made it possible to investigate interaction between the virus genotypes in virulent DWV populations, which showed that frequent recombination and mutation events allow the virus to escape specific antiviral RNAi. Tracing DWV variants also permitted novel insight into its transmission routes. Our studies showed that DWV type A is vectored by *Varroa* mites in a non-replicative manner, and also highlighted the importance of pupal cannibalism, which occurred in the -course of Varroa sensitive hygienic activity, for DWV circulation. We further demonstrated the possibility of using cloned DWV as an expression vector by designing DWV genomes with genes for green fluorescent protein and nanoluciferase reporters. The use of DWV with such reporter tags allows a large-scale screening of potential antiviral compounds.

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Dr. Ya Guo is an assistant professor at Northwest A&F University, China. With a background in insect virology and molecular biology, Dr. Guo is particularly interested in the mechanisms of molecular interaction between insect viruses and their hosts. Until last year, she held a postdoctoral appointment at the University of Florida, USA where she worked on honey bee viruses, with the goal of developing protective strategies against virus infections. She obtained her PhD in Entomology from Northwest A&F University, China. During her PhD, she worked for several months at the Boyce Thompson institute (BTI) at Cornell University on the functional role of host vesicular trafficking - related factors during baculovirus infection.

Virus-blocking peptides to mitigate virus burden in the honey bee

The Western honey bee (*Apis mellifera*) has been severely impacted by Varroa mites and associated viruses in recent years. The iflavirus, *Deformed* wing virus (DWV) and the dicistrovirus, *Israeli acute* paralysis virus (IAPV) are of particular concern in relation to colony losses. Following ingestion, DWV and IAPV are hypothesized to enter midgut epithelial cells by receptor-mediated endocytosis. By feeding adult honey bees on a phage display library, we identified a 7-amino acid, Bee midgut Binding Peptide (BBP2.1), which shared 67% and 86% identity with a region of the DWV and IAPV capsid proteins, respectively. This region is likely to be instrumental in virus interaction with the honey bee gut receptor. The binding of BBP2.1, and virus derived BBP2.1D^{WV} and BBP2.1^{IAPV} to honey bee gut proteins was confirmed. All three peptides compete with both IAPV and DWV virions for binding suggesting that the three peptides and the two viruses bind to the same protein or proteins. Feeding experiments indicated that ingestion of BBP2.1 reduced the movement of both IAPV and DWV from the honey bee gut into the body, supporting the utility of these peptides for peptide-mediated interference with virus infection.

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Lena is an evolutionary ecologist studying host-parasite interactions and the drivers of disease emergence. After her PhD at ETH Zürich (Switzerland) in 2006 on the genetic basis of disease resistance in bumblebees and post-docs at the Universities of Edinburgh and Cambridge on virus evolution in Drosophila, she obtained a Royal Society Dorothy Hodgkin fellowship in 2010 to study disease emergence in bees. From 2012 to 2018, she held this fellowship and a Senior Lectureship at the University of Exeter's Centre for Ecology and Conservation. In 2018, she became a Professor of Functional Biodiversitz at the Univerty of Ulm, Germany. She currently studies how the acquisition of vector-borne transmission affects viral evolution and epidemiology with an ERC consolidator grant.

Bee different: comparing virus diversity, prevalence and epidemiology in managed *A. cerana* and *A. mellifera* honeybees

Bee viruses not only offer a fascinating subject for studying the fundamental principles of host-pathogen interactions, but are of immediate importance for pollination services, food security and the beekeeping industry. In Asian apiculture, the Eastern honeybee *Apis cerana* was long dominant, with the Western honeybee *Apis mellifera*, now globally distributed, a late addition only introduced in the last centuries. Famously, *A. mellifera* acquired both *Nosema ceranae* and the ectoparasitic viral vector *Varroa destructor* from *A. cerana*, the latter leading to the re-emergence of Deformed Wing Virus, now the most iconic bee pathogen. However, in comparison to the well-studied *A. mellifera*, the epidemiology and diversity of bee viruses in *A. cerana* – not to mention the other Asian honeybee species – remain comparatively obscure. Here, we compare virus diversity in both species as well as the prevalence and epidemiological history of two virus complexes contrasting in their dependence on the Varroa vector – Deformed Wing Virus and Lake Sinai Virus – in both honeybee species. We show that these two honeybee species, despite their similarities in life history and management, show contrasting patterns in epidemiology. Particularly, we show that there is no evidence that *A. cerana*, despite being the source of Varroa, is the source of the current DWV pandemic.

BACTERIA DIVISION SYMPOSIUM : ANALYSIS OF VIP3A AND CRY PROTEIN MECHANISM OF ACTION

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Patricia Casino is a structural biologist with a background in Chemistry. Since 2016, she holds a tenure track position in Instituto Universitario Biotecmed at Department of Biochemistry and Molecular Biology of Universitat de València in Spain. Her group is interested in uncovering the molecular and structural basis of the signal transduction mechanism based on His-Asp phosphorelay systems present in bacteria. She aims to visualize the conformational changes related with phosphorylation, DNA binding and protein-protein interactions among others. Relevant publications are Huesa J et al. Nucleic Acids Res 2021; Nuñez-Ramírez R et al. Nat Commun 2020; Mideros-Mora C et al. Nat Commun 2020; Casino P et al. Nucleic Acids Res 2018; Casino P et al. Nat Commun 2014; Casino P et al. Cell 2009.

Mechanism of action of Vip3 proteins inferred from their structures

Co-authors : Patricia Casino, Rafael Núñez-Ramírez, Matthew J. Byrne, Juanjo Huesa, Matthew G. Iadanza, Yolanda Bel, Marcos Arribas Perez, Daniel P. Maskell, Rachel M. George, Emma L. Hesketh, Paul A. Beales, Marc D. Zack, Colin Berry, Juan Ferré, Rebecca F. Thompson and Ernesto Arias-Palomo

Bacillus thuringiensis Vip3 proteins have gained increasing interest since their use, in combination with Cry proteins, in biotech crops to control Lepidopteran pests. In the last year and a half, four research groups, working independently, have made important advances on the three-dimensional structure of Vip3A and Vip3B proteins, both by crystallography and cryo-electron microscopy. Overall, the results have revealed that the proteins are composed of five structural domains and have provided important clues on their possible roles. Vip3 proteins, in their protoxin form, assemble into a pyramid-shaped tetramer with the C-terminal domains exposed to the solvent and the N-terminal region folded into a spring-loaded apex. More importantly, after protease activation, the N-terminal region drastically remodels switching from the apex to the opposite side of the protein to form an extended needle of approx. 20 nm. The needle is composed of a tetrameric coil-coiled with an internal diameter large enough to accommodate divalent cations. Interestingly, recent cryo-electron tomography results show that the tip of activated Vip3Bb associates with lipid vesicles, further supporting the idea that this extension may play a crucial role in forming pores in the insect epithelial membrane. The elucidation of the 3D-structures of the protoxin and the activated form of Vip3 proteins has made a significant contribution to the understanding of how Vip3 proteins exert their insecticidal action and has provided molecular information on some unique features of these proteins, such as their oligomerization, cleavage without release of the N-terminal domain, and function of their five structural domains.

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I have been working with *Bacillus thuringiensis* ever since I joined David Ellar's lab in Cambridge in 1988. In 1995 I set up my own lab at Sussex University and have continued to work on Bt mechanism of action and insect resistance. I also look after the nomenclature system for pesticidal proteins from Bt and other bacteria.

Pesticidal protein mechanism of action – the importance of experimental verification.

It is widely accepted that Cry proteins (and many other pesticidal proteins) are pore-formers and that this is their primary mode of action. Exactly what kills the cell/insect depends on the extent of pore-formation and how the organism responds to this challenge. It is also generally accepted that efficient pore-formation requires prior binding of the pesticidal protein to a receptor either to concentrate the protein on the cell surface or to trigger oligomerization / membrane insertion. A number of more detailed models have been proposed which may be generally applicable or specific to a particular system. A main objective of this symposium is to consider the experimental evidence required to definitively characterize a mode of action model for a given system. This presentation will provide an introduction, and context, to the presentations that will follow and for a discussion session at the end.

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Dr. Bravo made PhD studies in Basic Biomedical Research (1989) in the National University of Mexico (UNAM). Since then she works in the Institute of Biotechnology, UNAM. Her research is focused to the study Cry and Cyt insecticidal toxins produced by Bacillus thuringiensis bacteria.

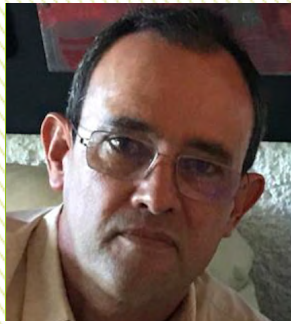
In 1991 she made a Postdoc in Plant Genetic Systems, Belgium and in 1995 another Postdoc in Institute Pasteur, France. She has done several short stays in EMBRAPA Brazil, Pionner Hi-Bred USA and Institute of Plant Protection, China.

Dr. Bravo has published 202 scientific articles, 33 book chapters, edited 4 books and has 8 patents. She received the research award of the Mexican Academy of Sciences, National University Award for young researchers, AgroBio México Award and Women in Science L'Oreal UNESCO Award.

Experimental evidence for Cry protein Mode of Action models

Bacillus thuringiensis Cry toxins (formerly 3D- Cry toxins) are a family of proteins whose members have shown toxicity against different insect orders or nematodes. Although the amino acid sequence identity could be low, all members share a similar protein fold composed of three distinct domains suggesting that the mode of action is conserved. These proteins have been widely used in biological control of insect pests worldwide.

Two different models for the mechanism of action of Cry toxins have been proposed, the signal transduction model (STM) or the sequential step model (SSM). In the case of STM, binding of Cry toxins to a membrane bound receptor, cadherin, triggers a G-protein dependent cascade that causes programed cell death. The SSM proposes that cell death is triggered by pore formation activity of the protein which results from sequential steps of the toxin, all of them assisted by receptor binding such as, concentration of toxin in the membrane, toxin oligomerization and oligomer insertion into the membrane. A fundamental difference between both models is that toxin oligomerization is a fundamental step for the SSM, while is not for the STM. We will summarize the experimental evidence that lead to both models and the experimental evidence that shows that toxin oligomerization is essential. Also, the role of ABCC2/3 in the mode of action of Cry proteins and its synergistic interaction with cadherin inducing Cry toxicity will be discussed in the context of the SSM.



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Since 1986 Dr. Mario Soberón has a position as researcher in the National University of Mexico (UNAM). He got his PhD in Basic Biomedical Research in the National University of Mexico (UNAM) 1989. Made two postdoc stays, one in Plant Genetic Systems, Belgium 1991 and other in Institute des Sciences Vegetales, France 1995. Besides he has made several research stays in EMBRAPA, Brazil, in Institute of Plant Protection, China and in Pionner Hi-Bred, USA. Dr. Soberón has described the molecular basis of insect specificity of Cry toxins from Bacillus thuringiensis, defined a distinct pathway for protoxin toxicity and the role of oligomerization in Cry toxin mode of action.

Dr. Soberón has published 175 research articles, 36 book chapters, edited two books and has 6 patents.

The activity of Cry protoxins

Bacillus thuringiensis bacteria synthesize insecticidal Cry toxins (formerly 3d-Cry toxins) that show specific toxicity against different insect orders or nematodes. All Cry toxins are synthesized as protoxins that are activated in the larval gut to yield a 60-65 kDa toxin fragment that is composed of three structural domains. However, two types of Cry protoxins have been described, the long protoxins of 130 kDa and the short protoxins of 70 kDa, both processed at the N-terminal removing 50 to a 100 amino acids. In the case of long protoxins, a C-terminal region of 65 KDa is also removed by proteolysis. The three-dimensional structure of the long Cry1Ac protoxin revealed four additional domains where domains V and VII showed a beta sandwich fold,

It was originally proposed that protoxin must be transformed into activated toxin fragment to execute toxicity. However, it was recently proposed a dual mode of action of Cry toxins, where protoxin exerts toxicity by an independent pathway of that of the activated toxin. We have shown that two distinct prepores are formed depending on whether the Cry1Ab protoxin or the corresponding toxin fragment is activated after cadherin binding. Furthermore, recent data showed that the C-terminal domain is also involved in binding to ALP and APN receptors, indicating an active role in toxicity. In this talk we will summarize the data suggesting that Cry protoxins exert toxicity by an independent pathway of that of activated toxin.

NEMATODE DIVISION SYMPOSIUM : ENTOMOPATHOGENIC NEMATODES OR SCAVENGERS : REVISITING THE EMERGING NEW NEMATODES CLASSIFIED AS EPN

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Researcher at the Institute of Entomology, Biology Centre of the Czech Academy of Sciences in Ceske Budejovice (https://www.entu.cas.cz/en/staff/profile/697-vladimirpuza/). He is focused on systematics and ecology of entomopathogenic, entomoparasitic and molluscoparasitic nematodes and their bacterial symbionts.

Entomopathogenicity and scavenging behaviour of Oscheius nematodes and their competition with entomopathogenic nematodes

In recent years, *Oscheius* nematodes have been proposed as potential entomopathogenic nematodes. Several studies also shown that these nematodes display scavenging behavior and can compete with entomopathogenic nematodes for insect host cadaver. The significance of entomopathogenic behavior and its distribution in the genus are not well understood, however, according to current knowledge, *Oscheius* nematodes seem to be rather scavengers being somewhere on the evolutionary trajectory to entomopathogenicity. In the present study, we evaluated entomopathogenic and scavenging behavior of *Oscheius myriophilus*, O. chongmingensis, *O. citri* and *O. siddiqi* using experimental insect hosts *G. mellonella* and *T. molitor*. In another series of experiments, we observed the interactions between *O. myriophilus* and *O. chongmingensis* and five entomopathogenic nematode species in live and freeze-killed G. mellonella larvae. The results support *Oscheius* nematodes as scavengers with only a limited ability to exploit live insect hosts. Both *O. myriophilus* and *O. chongmingensis* are able to compete with entompathogenic nematodes for insect host and the outcome depends on particular EPN species.

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Dr Giulia Torrini graduated in Phytosanitary Science and Technologies at the University of Florence in 2009 and obtained a PhD in Insect Science and Biotechnology at the University of Naples in 2015. Since 2012, she has been working in the Nematology Lab of the CREA Plant Protection and Certification.

She has been working on plant parasitic and entomopathogenic nematodes for biological control against harmful insects of agricultural and forestry crops. She is maintaining collections of nematodes of phytosanitary interest (Bursaphelenchus spp.) and entomopathogenic (Steinernematidae and Heterorhabditidae). She is the leader of the Task "Application of EPNs for biological control of P. japonica grubs in soil" for the H2020 project regarding IPM of the invasive Japanese Beetle, *Popillia japonica*.

The enigmatic status of *Oscheius onirici* (Nematoda: Rabditida)

Oscheius onirici was discovered in two different countries in 2013. The Italian isolate was collected in a karst cave of Tuscany (Central Italy) and it was characterized as an entomopathogenic nematode (EPN). In contrast, Swiss populations did not show entomopathogenic activity but behaved as facultative kleptoparasites, suggesting that this species should have been recharged as a scavenger rather than EPN. Our first hypothesis was that the cave nematode has adapted or specialized for survival in this environment and is likely in the process of evolving from a necromenic toward a more entomopathogenic status.

New strains of *O. onirici* were subsequently isolated from cranberry marshland in Wisconsin (USA) and laboratory bioassays have shown that this nematode is capable of infecting and killing, in less than 72 hours, various key pests that are particularly harmful to cranberry crops, such as the cranberry fruitworm, the sparganothis fruitworm, and the cranberry flea beetle. Moreover, *O. onirici* was tested again another pest of global importance for a wide variety of fruit crops, Drosophila *suzukii*, demonstrating that this nematode was able to effectively search within fruit substrate, find the fly larvae therein, and kill the insect before they could pupariate. Since different isolates of *O. onirici* have had different behaviours, how can we consider this nematode: a potential entomopathogen, having shown an interest in the biological control of different pests or a scavenger?

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Javad Karimi is an associate professor of insect pathology in the Ferdowsi University of Mashhad (FUM) (2008-now) which graduated from the University of Tehran (2007). His academic teaching and research interest focuses on insect pathogens as biocontrol agents with special reference to entomopathogenic nematodes (EPNs) (including their symbiotic bacteria) and insect parasitic nematodes

Biological and taxonomic characterization of a superior infective isolate of *Acrobeloids* spp.

Insect-associated nematodes are important neglected biocontrol agents, and accordingly, isolation and identification of the native field-collected nematodes are required to be used as a biological tool for controlling endemic insect pests. During a survey of entomopathogenic nematodes (EPNs) in various agricultural fields in Kerman region, a local insect-killing nematode was recovered from soil samples using the Galleria bait method. Morphological studies with light microscopy and scanning electron microscopy, as well as molecular analyses using 18S, ITS and 28S region of ribosomal DNA identified this isolates as *Acrobeloides* spp. The invasion, reproduction and foraging behavior of the native field-collected free living nematode were studied on two insect species. Despite the free living and parasitic behavior of this nematode, it causes infection in some insect pest's larvae and successfully recycles. Here we will discuss about challenges for clarifying *Acrobeloides* and other new emerging genus of insect associated rhabditids like *Oscheius* as insect pathogen. Comparison among real entomopathogen group and the complicated rhabditid genera from *Acrobeloides* and *Oscheius* in term of their potential role in biocontrol is another aspect of this paper.

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Dr. Raquel Campos-Herrera (ORCID: 0000-0003-0852-5269) is a Ramón y Cajal fellow (Government of Spain, 2018-23) at ICVV-CSIC. After her PhD at Universidad Complutense de Madrid (2006), she acquired >8 years' postdoctoral experience at University of Florida (USA), Agricultural Sciences Institute (CSIC, Spain), University of Neuchâtel (Switzerland), and University of Algarve (Portugal) supported by national/international fellowships. She authored more than 70 publications, including 65 IF-peer review (70% Q1)(H index = 20), and is the editor of the book 'Nematode Pathogenesis of Insects and Other Pests' (Springer, 2015). She serves as international expert for numerous project evaluation panels, supports various PhD students and perform outreach activities.

The cost of fighting for surviving in a complex world: entomopathogenic nematodes as scavengers

Entomopathogenic nematodes (EPNs) in the traditional families Steinernematidae and Heterorhabditidae are relevant and well-studied agents for the biological control of soil crop insect pests. Theoretically, their infective juvenile (IJ) stages existing in soils can act as entomopathogens colonizing live hosts or as facultative scavengers reproducing within dead hosts. Whether both paths are equally successful for EPNs is still poorly understood. Several studies proved that EPNs complete their life-cycle within freeze-killed hosts whose reproductive success depends on the host species, the number of IJs, and the presence of other scavengers, including free-living nematode (FLN) species in the genera Oscheius, Acrobeloides, and Pristionchus. In any case, the EPN offspring is commonly lower for IJs that reproduced as scavengers. Laboratory experiments also showed that high competitive pressures of FLNs reduced the virulence of IJs that emerged in low numbers from freeze-killed larvae. Alike, mix EPN and FLN progenies derived from infected larvae in soil baits often compromised EPN reproductive success. Some FLNs can even appear in nematode progenies of Koch's postulates tests. Whether this fact is due to FLNs behaving as entomopathogens or to the unnoticed presence of steinernematids or heterorhabditids are possibilities to consider for FLN species not included in the traditional EPN families. Further studies involving different soil-dwelling organisms, including the EPN symbiont bacteria, will be required to unravel the complex interactions occurring in soils and the diverse roles that each of them could play to increase their chances of success.

Dr Alder Dillman

PLENARY SYMPOSIUM - SIP AWARDEE SYMPOSIUM

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I started baculovirus research under the supervision of Dr. Susumu Katsuma, at Laboratory of Insect Genetics and Bioscience, Graduate School of Agricultural and Life Sciences, the University of Tokyo and received my doctoral degree in March 2021. I was working on host manipulation system of baculovirus, using Bombyx mori nucleopolyhedrovirus (BmNPV) and its host, Bombyx mori. My main projects were functional analysis of a baculovirus gene, *bv/odv-e26*, and development of novel method to analyse BmNPV-infected B. mori larvae using time-lapse camera, both of which are presented in the present meeting. This April, I moved to Institute of Chemical Research, Kyoto University as a postdoc, and work on giant viruses and their host protists.

bv/odv-e26 is required for virus-induced host behavioral manipulation in lepidopteran nucleopolyhedroviruses

Lepidopteran nucleopolyhedroviruses (NPVs) induce hyperactivity and modification of behavioral pattern in host insect larvae. Previous studies revealed that knockout of a gene specific to Group I NPV, *bv/odv-e26*, in Bombyx mori nucleopolyhedrovirus (BmNPV) induces lower host activation in larval Bombyx mori. As the knockout virus exhibited fast-killing phenotype, it was hypothesized that *bv/odv-e26* extends host survival time and keeps host locomotory activity at the late stage of infection. However, its function was unclear and controversial results were reported even in closely related NPVs. In the present study, we investigated the role of *bv/odv-e26* in NPV infection and its conservation. We examined behavior of B. mori larvae infected with a bv/odv-e26 knockout virus and found that *bv/odv-e26* is required for keeping larval physical condition at the late stage of infection. Transient expression of *bv/odv-e26* in cultured cells inhibited viral gene expression, which supports our hypothesis mentioned above. Furthermore, a synteny-based bioinformatic approach identified orthologous genes of *bv/odv-e26* in the genomes of most lepidopteran NPVs. Transiently expressed orthologue of Lymantria dispar multiple nucleopolyhedrovirus (LdMNPV) inhibited LdMNPV gene expression in *Lymantria dispar* cultured cells. Collectively, our results suggest that *bv/odv-e26* has a common function to optimize viral virulence for host behavioral manipulation by lepidopteran NPVs.

Dr Jörg Thomas Wennmann

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Dr. Jörg T. Wennmann is a research scientist at the Julius Kühn Institute, Institute for Biological Control in Darmstadt, Germany. He obtained his PhD degree from the University of Mainz (Germany), where he focused on the biology and diversity of baculoviruses isolated from cutworms under the supervision of Johannes Jehle. His postdoctoral was with Madoka Nakai at the Tokyo University of Agriculture and Technology in Tokyo, Japan, where he studied the genetic basis of occlusion body morphology of baculoviruses. In 2016, Jörg returned to Julius Kühn Institute where he became head of the laboratory for Molecular Insect Pathology and Bioinformatics. He is working on the molecular identification of insect pathogens especially baculoviruses, where he develops new approaches for the identification, differentiation and quantification of baculoviruses and their genotypes.

Deciphering the population structure of baculoviruses by nucleotide polymorphisms

High-throughput sequencing (HTS) technologies provide various possibilities to decipher the genetic variability within baculovirus isolates that reflect populations of genotypes that occur in different ratios due to replication, mutation and selection within their hosts. Todays, HTS techniques, such as Illumina or Nanopore Sequencing provide overwhelming read depth of sequencing information and thus novel tools for virus identification, characterization and functional studies. Baculovirus genome sequences can be used for species demarcation as well as for studying intra-species and intra-isolate sequence variability. Recently, we developed and applied new analyses tools, such as *bacsnp*, exploiting the information from single nucleotide polymorphisms (SNP) to study genetic heterogeneity in DNAseq and RNAseq data sets. SNP-specific analyses offer a broad range of application, including isolate identity, detection of contaminants and mixtures, population genetics and quality control in virus production. HTS methods will play a key role in dissecting in vivo infection and replication, not only in DNA sequencing but also in RNA sequencing, as the sequence data of the transcription can also be used to provide evidence that different virus genotypes of specific baculovirus are expressed within tissues of individual host larvae. The power of in depths HTS analyses will be presented on the example of various baculoviruses, including Cydia pomonella granulovirus, Bombyx mori nucleopolyhedrovirus and Lymantria dispar multiple nucleopolyhedrovirus, as well as Bombyx mori bidensovirus.

Dr Patricia Golo

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Since 2015, Patricia is a faculty member (professor) at the Department of Animal Parasitology from Federal Rural University of Rio de Janeiro, Brazil. She obtained her Ph.D. from the same University in 2014. Her Ph.D. focused on the biological control of cattle ticks using entomopathogenic fungi under the supervision of Dr. Bittencourt and Dr. Fernandes. During her Ph.D., Patricia was also a visiting scholar at Utah State University, supervised by Dr. Donald Roberts. Patricia applied for a public tender at UFRRJ and started her professional career at the veterinary institute. In 2016, Patricia lost her hearing, and despite this, she persisted in researching, advising, and teaching. Her research interests focus mainly on the use of entomopathogenic fungi to control ticks.

Fungi for tick control: what do we know and what do we need to know?

Co-autors : Patricia S. Golo[1], Jessica Fiorotti[1], Emily Mesquita[1], Thais A. Correia[1], Everton K.K. Fernandes[2], Donald W. Roberts[3], Vânia R.E.P. Bittencourt[1]

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Ticks are obligate blood-sucking parasites that cause direct deleterious effects both as parasites and as vectors of pathogens. Unfortunately, many tick-borne diseases do not have satisfactory treatments, and cannot be completely cured. To reduce tick-borne diseases is it necessary to reduce the abundance of ticks. The use of chemical acaricides is the most used method for tick control, but it has led to the emergence of resistant populations and raised concerns about human and environmental health. Using entomopathogenic fungi arises as a promising alternative when seeking for a safer and more sustainable method for tick control. The control of insect pests with micoinsecticides is successfully applied worldwide, but why the same doesn't happen with ticks? In fact, ticks are naturally less susceptible than insects to entomopathogenic fungi, however the reason why their susceptibility is higher is still unclear. It is suggested that the immune response of these arthropods, the composition of their cuticle, and possibly their microbiota influence this characteristic. In this presentation I intend to review the current knowledge about the use of entomopathogenic fungi to control ticks, particularly *Rhipicephalus microplus* and highlight the studies that have been conducted in our laboratory on the response of ticks after challenge with entomopathogenic fungi.

DIVISIONS-POSTERS & ORALS

BACTERIA DIVISION

PATHOGEN PHYSIOLOGY VIDEOS

The fate of bacteria of the Bacillus cereus group in the amoeba environment

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The Bacillus cereus group consists of several closely related species, including B. anthracis, B. cereus, and B. thuringiensis. Spores of these pathogenic bacteria are commonly found in the soil but evidence suggests that they are unable to grow in such a natural environment in the absence of nutrient input. Amoebas have been reported to be an amplifier for several species of pathogenic bacteria and their potential involvement to explain the large amount of B. thuringiensis and B. cereus spores in soil has been frequently proposed. Here, we studied the fate of Bacillus and amoebas when cultured together. We show that the virulence factors produced by B. thuringiensis and B. cereus do not affect the amoeba Acanthamoeba castellanii, which, on the contrary, can phagocytose and effectively digest vegetative Bacillus cells to grow and prevent the formation of cysts. Bacterial spores can germinate in the amoeba environment and the vegetative cells can then form chains or aggregates that appear to be less efficiently phagocyted by the amoeba. The use of transcriptional fusions between fluorescent reporter genes and stationary phase- and sporulation-specific promoters showed that the sporulation process occurs more efficiently in the presence of amoebas than in their absence. Moreover, our results showed the amoeba environment to promote spore germination and allow the bacteria to complete their developmental cycle. Overall, this study reports that the amoeba-Bacillus interaction creates a virtuous circle in which each protagonist helps the other to develop.

Together or separately? Effect of Bacillus thuringiensis spores and Cry toxins on Colorado potato beetle.

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Insect microbiota plays an important role in the life of insects, and can also be a method for pest control in agriculture. The gut microbiota could be an additional contributor to the virulence of Bacillus thuringiensis (Bt) bacteria. As a result of the studies, it was shown that the combined effect of Bt spores and Cry toxins leads to a synergistic effect in insect mortality compared to the separate treatments. In the early stages of pathogenesis, reactive oxygen species are released and lipid peroxidation is increased in the midgut (1.5 times) in variants treated Cry toxins ($p < 0.05$ compared with untreated). The bacterial infection with Bt in the larvae of the Colorado potato beetle Leptinotarsa decemlineata (CPB) leads to a qualitative and quantitative change in the composition of the intestinal microbiota, with a different effect of spores and crystalline endotoxin of bacteria on the main representatives of the microbial community. The development of a BT infection in CPB larvae leads to a decrease in the abundance of bacteria of the genus Pseudomonas in the intestinal microbiota 48 hours after treatment. Elevated level of genes expression of pattern-recognition receptor, serine proteases and prosaposin-like protein were detected in the midgut of infected CPB larvae. A search was carried out for symbionts in the intestines of the Colorado potato beetle with the aim of modifying them for the synthesis of RNA interfering constructs that block various physiological systems of insects.

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Cellular mechanisms causing midgut damage and insect death upon exposure to Bacillus thuringiensis insecticidal toxin

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Bacillus thuringiensis (Bt) produce a wide range of pore forming toxins used for targeted biocontrol of agricultural insect pests, yet our understanding of the mechanisms leading to death remain incomplete. The complex mode of action of Bt Cry1A toxins has been studied for decades using a diverse range of species and techniques, which have identified multiple protein receptors in the larval midgut, including ATP-dependent binding cassette transporter C2 (ABCC2). Here we compare the effects of exposure to Bt toxin Cry1Ac on a lepidopteran pest, the diamondback moth, and transgenic Drosophila that express the same moth ABCC2 receptor to compare and contrast their cellular responses and better understand how midgut cells die. Larvae of both species were exposed to LC50 doses of activated Cry1Ac toxin and midgut tissue analysed using transmission electron microscopy across a time series. Severe damage to the microvilli and rapid production of mitochondria were observed in both species, and proliferation of bacteria followed by and invasion of midgut cells was evident in diamondback moth. Parallel transcriptome sequencing using Illumina RNAseq confirmed increases in mitochondrial pathway genes, which may lead to oxidative stress in the cell. Genes involved in innate immunity and ion channels regulation were differentially expressed in both species after toxin consumption, however, genes involved in classic apoptosis and autophogy cell death pathways remained unaffected. These results suggest Bt toxins damage the plasma membrane, cause oxidative stress and weaken the innate immune system in diamondback moth, allowing opportunistic bacteria to proliferate and invade cells.

Xenorhabdus bovienii strain jolietti requires Type 6 secretion systems to kill closely related bacteria and colonize its nematode host

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Enterobacteria in the genus Xenorhabdus have a dual lifestyle: they are pathogens of many soil-dwelling insects and are mutualists of Steinernema nematodes. Infective juveniles (IJs), the only free-living stage in the nematodes' lifecycle carry Xenorhabdus while they search for a suitable insect host. Once inside an insect, Steinernema IJs release Xenorhabdus into the insect hemocoel. There, the bacteria replicate and produce many toxins that cause the death of the insect. Xenorhabdus bovienii strain jolietti (XBJ) (the symbiont of Steinernema jollieti) genome encodes two Type Six Secretion Systems (T6SSs) that can inject specific proteins directly into target cells, however their functions in XBJ lifecycle are unknown. We hypothesized that T6SSs play roles in XBJs insect virulence, interbacterial interactions, and nematode symbiosis. To elucidate the role of T6SSs in XBJ, mutants were created lacking the important structural and/or effector proteins, Hcp and VgrG genes from each T6SS cluster and evaluated for their ability to interact with bacteria, nematodes, and insects. Our results indicated that mutants lacking both VgrG genes and one of the Hcp (from T6SS-1) genes were unable to kill the prey. Additionally, mutants lacking both Hcp genes were unable to form biofilm. Moreover, IJs colonized with the mutant strain lacking both Hcp genes carried less bacteria compared to wild type. However, all the mutant strains resulted in full mortality of insects like the wild type. These results suggest that T6SSs of XBJ play roles in their antibacterial activity, biofilm formation, interactions with nematode host but not in insect virulence.

Xenorhabdus bovienii strain jolietti requires Type 6 secretion systems to kill closely related bacteria and colonize its nematode host

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While invertebrates rely exclusively on an innate immune system, some species display adaptive-like immune features, similar to the immune memory of vertebrates. Called "immune priming", the invertebrates' immune system can be stimulated during a first pathogenic infection, providing the host with protection (better resistance and/or tolerance) in the event of a second exposure to pathogens. This discovery, which appeared around the 1930's, opened several questions about the mechanistical and evolutionary ways of such innate immune ability. In this context, I conducted my PhD on the immune priming process of the terrestrial crustacean, Armadillidium vulgare, an organism presenting several suitable features for the emergence of this immune process. By performing experimental infections with the pathogenic bacterium Salmonella enterica, we demonstrated the existence of immune priming response in this species, both in term of survival improvement and cellular immune response of individuals previously primed with living or inactivated S. enterica. For the first time, we also explored the effect of age, gender, and the presence – or absence – of the endosymbiotic bacterium Wolbachia on the immune priming expression of A. vulgare. Overall, our results highlight that immune priming is influenced by these factors and their interactions, deepening our knowledge on invertebrates' protection against pathogens, especially in the light of host/pathogens/symbionts relationships.

Influence of arbuscular mycorrhizal symbiosis and nitrogen levels on the performance of Spodoptera exigua developing on maize: are effects mediated by a change of the insect gut microbiota?

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Nitrogen (N) is a critical chemical element for primary and secondary metabolism of plants, insects and microbes. Consequently, soil N availability may have significant bottom-up effects in multitrophic systems. Arbuscular mycorrhizal (AM) fungi are beneficial soilborne microorganisms that establish symbiotic associations with plants roots. AM symbiosis is known to influence plant nutrient uptake as well as plant defense responses to herbivorous insects. Similarly, phytophagous insects establish beneficial relationships with endosymbiotic bacteria that can influence immune and metabolic-related processes of their host, playing a key role in plant-insect interactions.

In this study, we investigate the effect of AM symbiosis in maize (Zea mays) on Spodoptera exigua larval performance under two different N fertilization levels. We hypothesize that AM colonisation on the one hand affects the performance of S. exigua by alteration of the larval endosymbionts, and on the other hand, influences how N can balance and/or perturbate this multiple partners interaction. Consequences of this symbiosis on the gut microbiota of insects is also characterized to decipher mechanisms underlying this complex interaction.

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The role of the microbiota in host resistance to pathogens in Galleria mellonella larvae.

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Currently, it is unclear to what extent the gut microbiota contributes to- or decreases host resistance to pathogens; and if there are some indigenous isolates of the microbiota that may improve the immune response to pathogens. Galleria mellonella (Lepidoptera: Pyralidae) larvae, an established model organism, will be reared in sterile conditions resulting in the production of axenic larvae without microbiota. By utilising Bacillus thuringiensis, and Metarhizium anisopliae, the role of the gut microbiota can be assessed via two routes of infection, in both axenic and conventional larvae. We have found that axenic G. mellonella larvae were more susceptible to orally administered B. thuringiensis than conventionally reared larvae. At a half-lethal dose, surviving conventional larvae can completely clear B. thuringiensis from their gut by 96 hours however; in surviving axenic larvae, the number of cells remains high. Our results also indicated that percentage mortality was always greater for the axenic larvae where 100% mortality was reached by 96h as compared to 50% mortality for conventional larvae. To further investigate the partnership between the microbiota and the immune response, several important immune response genes from the gut and fat body will be measured using qRT-PCR techniques and the composition and dynamics of the gut microbiota will be characterised during pathogen infection using 16S rRNA and the Illumina Miseq platform. There will also be an assessment of important life history traits to determine trade-offs corresponding to the presence or absence of the gut microbiota when challenged by a pathogen. These results will provide an understanding of how and to what extent the immune response is mediated by the microbiota, which can improve the potential for the development of probiotics and overall maintenance of insect health.

PATHOGEN PHYSIOLOGY
POSTERS

Effect of Diet and Antibiotic on the growth and fitness of laboratory reared Spodoptera exigua (Hübner)

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The success of insect mass rearing is largely dependent on an all-round optimal feed substrate. However, the dietary requirements for optimal growth, pupation, moth development, and survival for many laboratory-reared insects, are not well described. Moreover, it has been shown that diet contributes essential gut microbiota for the growth and survival of insect colonies but antibiotics, which are routinely used in mass rearing, have an effect on these. In this study, Spodoptera exigua Hübner (Lepidoptera: Noctuidae) was reared on two artificial diets, Alfalfa-based and wheat germ based in the presence and absence of Streptomycin antibiotic. Growth and fitness parameters recorded included: Growth rate (mg/day), larval period, percentage pupation, pupal weight, pupal period, percentage moth emergence and wing malformation and the survival rate. Overall, the colony performed better in Alfalfa based diet in all parameters measured, in two consecutive generations. Growth rate was highest in Alfalfa with antibiotic but this was not significantly different to the antibiotic treated group. In the absence of antibiotic, moths had a longer oviposition period although the pupal period was longer. Interestingly, there was a significantly lower rate of wing malformation in the Alfalfa based diet without antibiotic compared to the antibiotic treated group (3.75% and 32.25% respectively). These results suggest that the Alfalfa based diet likely contributes essential nutrients and key microbiota components for the growth and fitness of Spodoptera exigua and a deeper understanding of these interactions would be useful for the insect rearing industry.

Impact of probiotic bacteria on Tenebrio molitor fitness, gut microbial composition and susceptibility to Bacillus thuringiensis serovar tenebrionis and Metarhizium brunneum infections

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Tenebrio molitor (Coleoptera L.) the yellow mealworm is an insect model for infection and immunity studies and is used in mass-production of insects as feed and food. The industrial rearing of *T. molitor* on agricultural by-products may expose them to biocontrol residues, like environmental resistant Bacillus spores and fungal conidia, which could impact the fitness of *T. molitor.* Therefore, my PhD project deals with experiments analyzing different outcomes of single and co-infections of B. thuringiensis, and Metarhizium brunneum on the larval stages of T. molitor. Furthermore, as for other animals, the possible benefits of addition of probiotic bacteria to the feed will be analyzed. The pathogenicity of *B. thuringiensis serovar tenebrionis* (Btt) and *Metahrizium brunneum* KVL 12-30 are first tested by single infection on T. molitor to define LD25 and LD50. Then targeted co-infections will allow to determine additive, synergistic or antagonistic interactions between these pathogens. Alongside infections, feed uptake, growth rate etc. are recorded and gut microbiota composition is analyzed by 16s rRNA Mi-sequencing to measure how probiotic and pathogens modify the OTUs' composition. The hypotheses are: 1) *M. brunneum* and *Btt* have different mechanisms of infection, therefore dose and timing of pathogen exposure should influence the outcome 2) the presence of probiotics may help the insect to cope with the infection by improved immunity, by presenting a shorter period for pathogen clearance, by expressing better fitness performances etc. The poster will include the experimental set up and preliminary results.

PESTICIDAL PROTEIN MODE OF ACTION
VIDEOS

Investigating the importance of Cry2A activation in its activity toward Aedes aegypti

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Cry2A are 70kDa pesticidal proteins produced by *Bacillus thuringiensis* that often have dual activity against dipteran and lepidopteran species. It has long been known that while Cry2Aa has activity against the mosquito *Aedes aegypti* Cry2Ab does not. Several studies have indicated that Cry2Aa specificity towards *A. aegypti* is related to a putative receptor binding motif within Domain II. However, our previous work has shown that specificity is associated with four particular amino acids within domain I. We initially hypothesized that the N-terminal region containing these amino acids remains attached to the protein following activation and participates in receptor binding. Experimental data did not support this hypothesis but a more detailed analysis of the activation of the Cry2A proteins indicated that differences in the cleavage of Cry2Aa and Cry2Ab by A. aegypti gut enzymes associated with activity. Furthermore, the differences in cleavage associated with the aforementioned N-terminal amino acids. In this presentation we will present our model explaining the differential activity of Cry2Aa and Cry2Ab against *A. aegypti.*

Is activation of Bacillus thuringiensis Cry1Ia proteins necessary for toxicity?

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The *Bacillus thuringiensis* Cry1I proteins are structurally similar to other three-domain Cry proteins, while they are secreted at the stationary phase. Despite the considerable insecticidal activity of Cry1I proteins, their mode of action is not completely understood. In the present study, we investigated the toxicity of Cry1Ia protoxin and processed proteins (produced after trypsin or *Ostrinia nubilalis* midgut juice activation), as well as their oligomerization and binding properties. The Cry1Ia protoxin was found to be significantly more toxic for *O. nubilalis* than the processed proteins. The results of both *in vivo* and *in vitro* binding competition experiments showed that Cry1Ia protoxin and activated toxins bind specifically to the *O. nubilalis* BBMV, and share binding sites. Oligomerization studies promoted by *O. nubilalis* brush border membrane vesicles (BBMV) showed that neither Cry1Ia protoxin nor toxin form tetramers. Size exclusion chromatography assays revealed that the Cry1Ia protoxin remained as a dimer in solution while the protease-resistant cores were found as aggregates, dimers, and monomers. The *in silico* structural analyses indicate that Cry1Ia protoxin and activated proteins share Domains II and III, suggesting that, in Cry1Ia, these domains have a major role in specific binding, while domain I, which is mostly lost during the proteolysis, could be involved in toxicity. Consequently, unlike the mode of action proposed for the most known Cry proteins accumulated in crystal, the results showed that the activation step is not necessary for exhibiting the highest Cry1Ia toxicity.

Hetero-oligomerization of *Bacillus thuringiensis* Cry1A proteins enhance binding to the ABCC2 transporter of *Spodoptera exigua*

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The ABC transporters are proteins that can act as surrogate receptors for Cry proteins from Bacillus thuringiensis (Bt) in the midgut of different insects. For the beet armyworm, Spodoptera exigua, ABCC2 and ABCC3 have been found to interact with Cry1A proteins, the main insecticidal proteins used in Bt-crops, as well as Bt-based pesticides. The ABCC2 has shown to have specific binding towards Cry1Ac and is involved in the toxic process of Cry1A proteins, but a clearer understanding on the role of this transporter and how relates with the proteins is needed. Here, we have characterized the interactions between the SeABCC2 and the main proteins that bind to the receptor. By labelling the Cry1Aa protein, we have found that virtually all of the binding is in an oligomeric state, a conformation that allowed higher levels of specific binding that could not be achieved by the monomeric protein on its own. Furthermore, we have observed that Cry1A proteins can hetero-oligomerize in the presence of the transporter, which is reflected in an increase in binding and toxicity to SeABCC2-expressing cells. This synergism can be one of the reasons why B. thuringiensis co-expresses different Cry1 proteins that can apparently have similar binding preferences. The results from in vitro competition and ex vivo competition showed that Cry1Aa, Cry1Ab and Cry1Ac share functional binding sites. By using Cry1Ab-Cry1Ac chimeras, the presence of domains I and II from Cry1A proteins was revealed to be critical for oligomer formation.

Specific binding of radiolabeled Vip3Af to brush border membrane vesicles from *Spodopterda* spp. and determination of the domains involved

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The understanding of mode of action of Vip3 proteins from *Bacillus thuringiensis* is crucial to guide their application, deserving an especial interest the role of specific membrane receptors on their specificity. In this study, we have set up the conditions to analyze the specific binding of ^{125}I-Vip3Af to *Spodoptera frugiperda* and *Spodoptera exigua* brush border membrane vesicles (BBMV). Heterologous competitions revealed that Vip3Aa shares the same binding sites with Vip3Af, but that Vip3Ca does not recognize all of them. As expected, Cry1Ac and Cry1F did not compete with Vip3Af binding sites. By trypsin treatment of selected alanine-mutants, we were able to generate truncated versions of Vip3Af. Their use as competitors with ^{125}I-Vip3Af indicated that only those molecules containing domains I to III (DI-III and DI-IV) were able to compete with the trypsin-activated Vip3Af protein for binding, and that molecules only containing either domain IV or domains IV and V (DIV and DIV-V) were unable. These results were further confirmed with competition experiments using ^{125}I-DI-III. In addition, the truncated protein 125I-DI-III also bound specifically to Sf21 cells. Cell viability assays showed that DI-III and DI-IV were as toxic to Sf21 cells as the activated Vip3Af, suggesting that

PESTICIDAL PROTEIN MODE OF ACTION
POSTERS

Establishing the role of glycans and lipids in the mechanisms of Tpp1/Tpp2 (Bin) toxin.

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Tpp1/Tpp2 (formerly BinA/BinB) toxin is a two-part (binary) toxin of the toxin_10 family composed of Tpp1 and Tpp2 which, combined, target the larvae of Culex and Anopheles mosquitoes. In addition to toxicity against target insect larvae and cells, Tpp1/Tpp2 toxicity against a range of mammalian cells has also been reported even in the absence of the putative Bin receptor (Cqm1). Previous studies, with invertebrate-active pore forming toxins (Cry5B and Cry14A), have identified that glycan moieties can mediate toxicity through facilitating toxin/receptor binding, and indeed some works suggests Tpp1 may also have the ability to interact with L-fucose, L-arabinose, and glycoproteins.

We have investigated the roles of lipids and glycans in Tpp1 and Tpp2 toxin binding and efficacy in various insect and mammalian cells lines – with a focus on its known target species, *Culex quinquefasciatus.* Preliminary findings indicate Tpp1/Tpp2 does not interact with sugars previously associated with insecticidal protein action (e.g GalNAc, GlcNAc), and drug-mediated reduction of N-glycosylation does not impact toxicity in cell lines. Initial studies using total lipid extracts from C. quinquefasciatus larvae and cell lines suggest Tpp1 and Tpp2 bind several lower phase lipids (simple glyco and other non-polar lipids), and upper phase lipids (hydrophilic, generally with more polar carbohydrate structures). Tpp1 and Tpp2 also bind lipid species extracted from a range of non-target insect species and mammals indicating this, in itself, is not enough to elicit cell death. Future work will focus on

RECEPTOR AND RESISTANCE
VIDEOS

Cadherin fragment from *Spodoptera exigua* enhances Cry1A toxicity to *Grapholita molesta*

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The arisen of insect resistance to chemical pesticides as well bio-insecticides is one of the most serious concerns in agriculture and pest management programs. *Bacillus thuringiensis* (Bt), a gram-positive bacterium, is nowadays the most commercialized bio-pesticide used worldwide. During the vegetative and sporulation phases, Bt produces different proteins toxic to many insect orders, either secreted (Vip and Sip) or accumulated in the crystals (Cry and Cyt). Diverse strategies have been developed to enhance the toxicity of Bt toxins to delay insect resistance, including the addition of cadherin fragments, as a synergistic agent. In this study, we have used two different cadherin fragments, one from a lepidopteran species, *Spodoptera exigua* (rSecad1bp), and the other from a coleopteran species, Tenebrio molitor (rTmCad1p), to test their synergistic effect on the toxicity of Cry1Aa, Cry1Ab and Cry1Ia on the lepidopteran pests *S. exigua* and *Grapholita molesta*. Our results showed that rSeCad1bp had no effect on its source insect species (*S. exigua*), but it increased about 2,7 folds toxicity potency of Cry1Aa and Cry1Ab on G. molesta. No other synergistic or antagonist effects were observed. The enhancement of Cry1A toxicity by cadherin fragments has been associated with an increase in oligomer formation, but this fact was not evidenced in our experiments. In conclusion, we found that a fragment of cadherin from a lepidopteran insect species enhances the toxicity of Cry protein on another insect species. Further experiments are required to shed light on the possible use of cadherin fragments in the pest resistance management.

Bt resistance-associated alteration of aminopeptidase N (APN) gene expression is independent of the *ABCC2* gene in *Trichoplusia ni*

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Development of resistance to toxins from *Bacillus thuringiensis* (Bt) in insect pests threatens the sustainable application of Bt in agriculture. The resistance to the Bt-toxin Cry1Ac in the cabbage looper, *Trichoplusia ni*, has been identified to be associated with altered expression of the aminopeptidase-N (APN) genes APN1 and APN6 in the larval midgut and mapped to the locus of the ATP-binding cassette (ABC) transporter ABCC2 gene. Whether the altered expression of *APN1* and *APN6* genes in Cry1Ac-resistant T. ni is regulated by or associated with the *ABCC2* gene requires to be understood. In this study, we investigated the association of the altered expression of *APN1* and *APN6* genes with the *ABCC2* gene in *T.* ni. Our genetic linkage analysis identified that the alteration of APN gene expression in Cry1Ac resistant T. ni is conferred by a gene independent of the ABCC2. Proteomic and genetic analysis of the midgut brush border membrane APNs showed that the down-regulation of APN1 and up-regulation of APN6 are independent of the ABCC2 gene mutation. Furthermore, knockout mutations of the *ABCC2* gene in T. ni were found to have no effects on the expression of *APN1* and *APN6* genes in *T. ni*. Therefore, the Cry1Ac resistance-associated alteration of

Identification of a new Cry1Ac resistance gene in *Heliothis virescens*

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The CP73 strain of *Heliothis virescens* (recently renamed as Chloridea virescens) resulted from laboratory selection with Cry1Ac of a field-collected susceptible population from North Carolina (Gould et al. 1992 PNAS USA 89: 7896). It attained 50-fold resistance to Cry1Ac, but also 20-fold resistance to Cry2A, which was not expected because the two toxins were believed to have independent modes of action. Further genetic analysis indicated a single major autosomal locus (*BtR-5*) for Cry1Ac resistance, which however made no detectable contribution to Cry2A resistance (Gahan et al. 2005 J. Econ. Entomol 98: 1356). *BtR-5* is genetically unlinked to every known Cry1A or Cry2 resistance mechanism in Lepidoptera, including modified cadherins, ABC transporters, alkaline phosphatase, aminopeptidase N, and tetraspanin. Individuals homozygous for the resistant allele at BtR-5 and for the deletion of the 12-cadherin domain protein have a resistance level about equal to the additive prediction. Further progress in identifying the molecular identity of *BtR-5* will be reported. (Supported by the Max-Planck-Gesellschaft.)

Comparison of *in vitro* and *in vivo* binding sites competition of *Bacillus thuringiensis* Cry1 proteins in two important corn pests

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Binding sites models, derived from *in vitro* competition binding studies, have been widely used for predicting potential cross-resistance among insecticidal proteins from *Bacillus thuringiensis*. However, because some discrepancies have been found between the binding data and the observed cross-resistance patterns in some insect species, an *in vivo* approach has been applied for the competition studies to determine the functional relevance of the shared binding sites as determined by *in vitro* competition assays. By using Cry disabled proteins as competitors in mixed proteins overlay assays we have been able to determine the preference of Cry1Ab, Cry1Fa and Cry1A.105 proteins for shared binding sites in vivo in two important corn pests, *Ostrinia nubilalis* and *Spodoptera frugiperda*. Our data support that both methods, the *in vivo* and *in vitro* approaches, can provide useful information to better ascertain whether different Cry proteins share binding sites and, consequently, cross-resistance due to binding site

In vivo competition assays between Vip3 proteins confirms the existence of shared binding sites among them in *Spodoptera littoralis* with different relevance on the toxicity

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Due to their different specificity, the use of Vip3 proteins from *Bacillus thuringiensis* in combination with the conventionally used Cry proteins in crop protection is being essential to counteract the appearance of insect resistance. Therefore, understanding the mode of action of Vip3 proteins is crucial for their better application, with special interest on the binding to membrane receptors as the main step for specificity. Derived from *in vitro* heterologous competition binding assays using ^{125}I-Vip3A and other Vip3 proteins as competitors, it has been shown that all Vip3 proteins share receptors in *Spodoptera frugiperda* and *Spodoptera exigua* brush border membrane vesicles (BBMV). In this study, using ^{125}I-Vip3Aa, we have first extended the *in vitro* competition binding site model of Vip3 proteins to *Spodoptera littoralis*. With the aim to understand the relevance of this binding to midgut sites observed *in vitro* to the insecticidal activity of these proteins, we have performed *in vivo* competition assays, with S. littoralis neonates, using disabled (non-toxic) Vip3 proteins as competitors for blocking the toxicity of Vip3Aa and Vip3Af. The results of the *in vivo* competition assays confirm the occurrence of shared binding sites. Discrepancies between the two approaches can be explained by the role played by gut proteases in the conversion of the protoxin form into the active one.

Functional validation of DvABCB1 as a receptor of Cry3 toxins in western corn rootworm, *Diabrotica virgifera virgifera*

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Western corn rootworm (WCR), Diabrotica virgifera virgifera, is a major pest in the main corn growing areas of North America and in parts of Europe. Resistant populations of WCR populations to Bacillus thuringiensis (Bt) toxins have been reported, raising concerns over the continued efficacy of these traits in WCR management. A thorough understanding of the mode of action of these Bt proteins is important for effective resistance management. Although different midgut proteins have been identified as Bt receptors in lepidopteran insects, identification of receptors in WCR has been limited with no reports of functional validation. We show that heterologous expression of DvABCB1 in Sf9 or HEK293 cells conferred sensitivity to Cry3A toxins, but not other WCR-active toxins. Functionality was further validated using knockdown of DvABCB1 by RNAi which rendered WCR larvae insensitive to a Cry3A toxin. However, silencing of DvABCB2, which is highly homologous to DvABCB1 at the amino acid level, did not reduce the sensitivity of WCR larvae to a Cry3A toxin. Finally, reduced expression and alternatively spliced transcripts of DvABCB1 were identified in a mCry3A-resistant strain of WCR previously shown to exhibit reduced binding of a Cry3A toxin. Our results provide the first clear demonstration of a functional receptor in the molecular mechanism of Cry3A toxicity in WCR and confirmed its role in the mechanism of resistance in a mCry3A resistant strain of WCR.

Resistance to Bt Cry1Ac in *Trichoplusia* ni is conferred by multiple gene mutations

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The resistance to Bt toxin Cry1Ac in greenhouse populations of *T. ni* has been previously mapped to the *ABCC2* gene locus. The mapped Cry1Ac resistance locus was a 540 kb region on Chromosome 15. In this study, the Cry1Ac resistance was further finely mapped in the *T. ni* genome. By whole genome resequencing of individuals from the Cry1Ac resistant *T. ni* strain, cDNA sequencing of candidate genes in the finely mapped resistance locus and genomic DNA sequencing of the identified mutation region, the specific mutation associated with Cry1Ac in T. ni was identified to be in the *ABCC2* gene. To examine the association of *ABCC2* mutation with Cry1Ac resistance, *T. ni* mutant strains were generated by CRISPR/Cas9 mutagenesis, and the results from bioassays of the mutants with Cry1Ac confirmed the functional role of *ABCC2* in the toxicity of Cry1Ac in *T. ni*. However, loss of function mutations in ABCC2 did not reproduce the same level of resistance as observed in the Cry1Ac resistant *T. ni* strain. Genetic analysis of the Cry1Ac resistant *T. ni* with CRISPR mutant strains indicated that the high level Cry1Ac resistance in the greenhouse-derived resistant T. ni strain is conferred by multiple gene mutations.

The Silkworm ABCC transporters are involved in susceptibility difference for each *Bacillus thuringiensis* Cry1Ab, Cry1Ac and Cry1Fa toxin

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Bacillus thuringiensis produces insecticidal Cry toxins that have been used worldwide to control insect-pests in agriculture. Recent reports have suggested that ATP-binding cassette transporter subfamily C2 (ABCC2) and Cadherin-like receptor (BtR175) play important roles for conferring susceptibility to Cry1A toxin. However, it is still unclear which receptor is involved in susceptibility of Bombyx mori larvae to various Cry toxins. To determine susceptibility determining roles of the BmABCC2 and BmABCC3 against to Cry1Ab, Cry1Ac and Cry1Fa, BmABCC2 or BmABCC3 single knockout Bombyx mori (C2-KO or C3-KO) and double knockout (C2C3-KO) were used in diet-overlay bioassays. To Cry1Ab and Cry1Ac, C2-KO and C2C3-KO strains represented high resistance, however C3-KO strains showed no resistance. To Cry1Fa, C2C3-KO strains showed significant resistance, however C2-KO and C3-KO strains showed no resistance. These results indicated that to Cry1Ab and Cry1Ac BmABCC2 makes higher level contribution in determining the susceptibility than BmABCC3, but to Cry1Fa BmABCC2 and BmABCC3 make contribution of the same level. Then, to confirm whether the ABCC transporters and BtR175 exert a synergistic effect for susceptibility of Cry toxins, we expressed BmABCC2 or BmABCC3 and co-expressed BmABCC2/3 and BtR175 in Sf9 cells. The results showed BmABCC2 and BmABCC3 are functional receptors of Cry1Ab, Cry1Ac and Cry1Fa at different level in conferring susceptibility, and co-expression caused a higher susceptibility to each toxin. Furthermore, SPR analysis was used to confirm that the binding affinity between ABCC transporter and toxins is involved in the susceptibility of Cry toxins.

RECEPTOR AND RESISTANCE
POSTER

Alteration of a Cry1A shared binding site in a laboratory selected strain of *Ostrinia furnacalis* resistant to Cry1A proteins

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The Asian corn borer Ostrinia furnacalis (Guenée, 1854) is a highly damaging pest in Asia, and larvae of this species feed mainly from corn crops. An effective control of corn lepidopteran pests in other parts of the Globe is accomplished by the use of Bt corn expressing the Cry1Ab insecticidal protein from *Bacillus thuringiensis* (Bt). This protein is highly effective against *O. furnacalis*. To determine the suitability of this technology for the future control of this pest, understanding of the potential to develop resistance to Cry1Ab and the basis of cross-resistance to other Cry1 proteins by alteration of shared binding sites is needed. In the present study we have shown the binding of Cry1A proteins to brush border membrane vesicles from two *O. furnacalis* strains, one susceptible (BtS) and one resistant (AbR), which had been selected with the Cry1Ab protein. The resistant insects were cross-resistant to Cry1Aa and Cry1Ac. Binding assays with radiolabeled Cry1Ab and Cry1Aa and susceptible insects showed that Cry1A proteins had shared binding sites. The resistant insects showed a one half reduction in the specific binding of both Cry1Ab and Cry1Aa, suggesting that part of the binding sites were lost. This was confirmed by competition binding assays in which results indicated that resistant insects had lost one shared binding site. Therefore, *O. furnacalis* has several binding sites that are shared by Cry1Aa, Cry1Ab, and Cry1Ac, and the resistant strain has lost one of them.

STRAINS AND PROTEINS
VIDEOS

Peptide mediated enhancement of a bacterial ETX-MTX pesticidal protein for suppression of the southern green stink bug, *Nezara viridula*

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Damage resulting from stink bug feeding accounts for significant agricultural losses on a global scale. Having demonstrated retargeting of a *Bacillus thuringiensis* (Bt) cytolytic toxin for aphid control, we adopted a similar approach to enhance the efficacy of the Etx/Mtx pesticidal protein ARP147 for use against the southern green stinkbug, *Nezara viridula*. Gut binding peptides were isolated by screening a phage display library either ex vivo for binding to brush border membrane vesicles, or in vitro for binding to recombinant *N. viridula* aminopeptidase N (NvAPN). The peptide selected from the ex vivo screen, NvBP1 bound to alpha amylase N4. Following confirmation of binding and binding specificity, peptides NvBP1 and ABP5 (seven amino acids) were used to modify ARP147-maltose binding protein (MBP) fusions by addition to- or replacement of- existing amino acid sequence. Binding of modified ARP147-MBP to BBMV varied according to both peptide and site of peptide insertion based on pull down and MST assays. The addition of gut binding peptides to ARP147-MBP significantly increased toxicity against *N. viridula* nymphs, but relative binding was not predictive of enhanced insecticidal activity. Constructs modified for increased alpha amylase binding showed greater toxicity than those modified for increased APN binding, possibly reflecting the higher abundance of alpha amylase in the anterior gut of *N. viridula*. This peptide modification strategy will allow for transgenic plant-mediated management of *N. viridula* to mitigate economic loss associated with this important pest.

A novel insecticidal protein is toxin to Ostrinia furnacalis and Agrotis ipsilon

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Transgenic crops producing insecticidal proteins of *Bacillus thuringiensis* (Bt)could well control pests. Asian corn borer (*Ostrinia furnacalis*) and Black cutworm (*Agrotis ipsilon*) are two major lepidopteran pests of corn. In China, *Ostrinia furnacalis* caused loss of yield up to 9 million tons annually, *Agrotis ipsilon* is an occasional pest, which is mainly damage to maize seedlings. Here we identified a novel insecticidal protein Bt0416, with significant toxicity against *Ostrinia furnacalis* and *Agrotis ipsilon*, the toxin activities are 38% and 42% at 1μg/cm2 protein concentration respectively. Bt0416 is a 42KDa member of the toxin_10 superfamily, sharing low identity (<30%) with other known insecticidal of Bt. Crystal structure of Bt0416 have determined, the resolution is 1.6 Å. We are making Bt0416 mutants in order to enhance the toxicity to develop Bt crops, through carrying out directed evolution base on protein structure. Because of the huge difference in structure, Bt0416 will not easy produce cross-resistance with other commercial insecticidal proteins, such as Cry1 toxins. It has brilliant prospect in development of Bt corns to target *Ostrinia furnacalis* and *Agrotis ipsilon*.

Occurrence of endophytic *Bacillus thuringiensis* strains in wild vegetation plants

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Bacillus thuringiensis (Bt) is a gram-positive bacterium that has been isolated from a multitude of sources such as soil, water, dust, dead insects and, recently, from internal plant tissues. To date, this habitat has been studied mainly in plants of agricultural interest; however, very little is known about Bt strains isolated from wild plants. To determine the frequency that this bacterium can be found in nature as an endophyte, 110 plants of different species, including mostly wild as well as some ornamental plants, were collected to analyze their bacterial endophytic composition. Strains showing parasporal bodies of any sort at the sporangium state were isolated and characterized morphologically, toxicologically, and molecularly. As a first systematic search, it was evident that *B. thuringiensis* can be found as endophytic bacteria in nature, with a 20% frequency. Characteristics such as the morphology and composition of amorphous crystals were recurrent among the most of these strains, although some showed the typical bipyramidal crystal. Flagellin gene sequencing identified stains within serotypes *nigeriensis, amagiensis, kurstaki, kyushuensis, darmstadiensis,* and *thuringiensis*. The 16S sequencing indicated an ID within the *cereus* group in all the isolates. Two of these strains showed some toxicity towards lepidopteran species. Interestingly, half of the total isolated strains were similar to the species *B. paranthracis*; although they showed some sort of parasporal crystals in their sporangium. This result could indicate that it is a new serotype that may be closely related to endophytic habits.

Effect of the Cry10Aa protein from *Bacillus thuringiensis* expressed in *Coffea arabica* plants on the coffee berry borer *(Hipothenemus hampei)*

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Bacillus thuringiensis has been widely used in integrated pest control programs in many crops worldwide. Its efficiency has decreased carbon emissions and the use of highly toxic synthetic insecticides. This report presents an efficient protocol for stable genetic transformation of coffee plants expressing the Cry10Aa protein from *B. thuringiensis* to induce coffee berry borer (CBB) resistance using the biolistics technique for genetic transformation of somatic embryos. Resistant coffee trees to CBB were produced faster than other reported techniques, spending only eight months in the regeneration period using highly embryogenic cell lines and adequate somatic embryo maturation to yield successful conversion to plants. A high efficiency of genetic transformation of 16.7% was obtained, according to the number of embryogenic aggregates and transgenic lines developed. Hygromycin-resistant embryogenic lines, green fluorescent protein expression, quantitative analyses of expressed Cry10Aa protein by mass spectrometry, Western blot, ELISA, and Southern blot analyses, showed stable genetic transformation of coffee plants. Two-year old transgenic trees developed fruits which demonstrated expression of the *B. thuringiensis* toxin ranging from 3.25 to 13.88 μg/g fresh tissue. Bioassays with 1st-instar larvae and adults CBB on transgenic fruits induced mortalities between 85 and 100% after 10 days. Additionally, transgenic fruits showed less than 9% seed damage, in average, as compared to 100% of control fruits. This is the first report of a stable transformation and expression of the Cry10Aa protein in coffee plants with the potential to control the CBB.

STRAINS AND PROTEINS
POSTERS

The rapid evolution of resistance to Vip3Aa insecticidal protein in *Mythimna separata* (Walker) is not related to altered binding to midgut receptors

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Laboratory selection for resistance of field populations is a well-known and useful tool to understand the potential of insect populations to evolve resistance to insecticides. It also allows us to study the mechanisms by which insects developed resistance to shed light on the mode of action and optimize resistance management strategies. In here, a field population of *Mythimna separata* was subjected to laboratory selection with either Vip3Aa, Cry1Ab or Cry1F insecticidal proteins from *Bacillus thuringiensis*. The population rapidly evolved resistance to Vip3Aa reaching, after 8 generations, a level of >3061-fold resistance compared with the unselected insects. In contrast, the same population did not respond to selection with Cry1Ab or Cry1F. The Vip3Aa resistant population did not show cross-resistance to either Cry1Ab or Cry1F. Radiolabeled Vip3Aa was tested for binding to brush border membrane vesicles from larvae from the susceptible and resistance insects. The results did not show any difference between both insect samples. Our data, along with previous results obtained with other Vip3Aa-resistant populations from other insect species, suggest that altered binding to midgut membrane receptors is not the main mechanism of resistance to Vip3Aa.

Crystal Structure of Lysinibacillus sphaericus Tpp49 using Serial Femtosecond Crystallography

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Lysinibacillus sphaericus is an entomopathogenic bacterium with the ability to produce crystal proteins which exhibit mosquitocidal activity. The two-part Cry48/Tpp49 pair is composed of Cry48, belonging to the 3-domain family of Cry proteins, and Tpp49 (formerly Cry49), belonging to the family of Toxin-10 Pesticidal Proteins. Mosquito bioassays have revealed activity against Culex mosquitoes and that both proteins are required for activity, with optimal toxicity arising from a ~1:1 ratio. Importantly, studies demonstrating Cry48/Tpp49 toxicity to Tpp1/Tpp2 (formerly BinA/BinB)-resistant larvae have highlighted distinct differences between the mode of action of the Cry48/Tpp49 toxin and the Tpp1/Tpp2 toxin, the latter of which is the major insecticidal factor used in mosquitocidal biolarvicides. These differences highlight the potential of the Cry48/Tpp49 toxin for managing mosquito resistance to the Tpp1/Tpp2 toxin.

Here, we use serial femtosecond crystallography combined with an X-ray free electron laser to determine the Tpp49 structure from natural crystals isolated from spores. Diffraction data extended to 2.2 Å and revealed the presence of a homodimer with a large intermolecular interface, similar to that of the Tpp1/Tpp2 binary pesticidal proteins in their natural crystals. Within each monomer, two distinct domains exist: An N-terminal β-trefoil domain and C-terminal pore forming domain with a topology characteristic of the Aerolysin family of pore forming toxins. Understanding the structure-function relationship of the Cry48/Tpp49 toxin will be crucial for understanding the mechanism of this toxin fully.

Streamlined phage display library protocols for identification of insect gut binding peptides highlight peptide specificity

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Short peptides that bind to the insect gut can be used in insects both as pathogen transmission blocking agents, and as artificial anchors for increased toxicity of bacteria-derived pesticidal proteins. Traditionally, screening of a phage display library resulted in identification of phage clones displaying enriched peptides that were sequenced by Sanger sequencing. Here we present a streamlined protocol for identification of insect gut binding peptides, using insect-appropriate feeding strategies, with next generation sequencing and tailored bioinformatics analyses. The bioinformatics pipeline is designed to eliminate poorly enriched and false positive peptides, and to identify peptides predicted to be stable and hydrophilic. In addition to developing streamlined protocols, we also sought to address whether candidate gut binding peptides can bind to insects from more than one order, which is an important consideration for safe, practical use of peptide-modified pesticidal proteins. We therefore screened phage display libraries for peptides that bind to the gut epithelia of two pest insects, the Asian citrus psyllid, Diaphorina citri (Hemiptera) and beet armyworm, *Spodoptera exigua* (Lepidoptera), and one beneficial insect, the western honey bee, *Apis mellifera* (Hymenoptera). While unique peptide sequences totaling 13,427 for *D. citri*, 89,561 for S. exigua and 69,053 for *A. mellifera* were identified from phage eluted from the surface of the insect guts, final candidate pools were comprised of 53, 107 and 1423 peptides, respectively.

DIVISION OF BENEFICIAL INVERTEBRATES

DISEASES OF BENEFICIAL INVERTEBRATES
VIDEOS

The enemy that lurks: egg-predators of the Caribbean spiny lobster

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The Caribbean spiny lobster (*Panulirus argus*) fishery generates almost 1 billion dollars in revenue throughout the Caribbean, including Saint Kitts and Nevis. Nemertean worms in the *Carcinonemertes* genus are egg-predators of ovigerous decapod crustacean hosts, affecting broods and negatively impacting some crustacean fisheries. In 2017, the novel *Carcinonemertes conanobrieni* was reported in P. argus from Florida and subsequently reported in Colombia. Two *Carcinonemertes* spp. were detected in P. argus on Saint Kitts and Nevis using gross, microscopic, histological and molecular analyses. They were distinguished from each other due to differences in development on the host, egg mass morphology, and COI DNA sequences. Phylogenetic and genetic distance analyses revealed the presence of *C. conanobrieni* and an undescribed species. *Carcinonemertes conanobrieni* was found in broods of ovigerous P. argus and lays eggs in a string-like arrangement. The undescribed *Carcinonemertes* sp. was found in the gills of male and female P. argus (72 out of 320; 23%), and egg masses in host broods are more spherical. *Carcinonemertes* spp. were found in 27 of 31 (87%) of ovigerous P. argus. This is the third report of *C. conanobrieni* in the geographical range of P. argus and the first report of the undescribed *Carcinonemertes* sp.

Identification and quantification of entomopathogenic viruses in reared crickets

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Interest in developing sustainable protein alternatives for human consumption, both direct and indirect via animal feed, has gained incredible momentum. One such alternative is insect-derived protein and crickets (family: Gryllidae) are an especially popular group of edible insects due to their nutritional quality and palatability. However, this emerging insect crop has been severely impacted by microbial entomopathogenic infections of which we know very little about. Here, I identified and quantified pathogenic viruses isolated from colonies of Gryllodes sigillatus and Acheta domesticus crickets, using various molecular and genomic techniques. I will discuss these results and highlight the future considerations necessary for ensuring the health of farmed insects.

Epigenetic and metabolomic changes in hemocytes underlie innate immune memory in the vector snail Biomphalaria glabrata

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Discoveries made over the past ten years have provided evidences that invertebrate antiparasitic response may be primed in a sustainable manner, leading to the failure of a secondary encounter with the same pathogen. This phenomenon called "immune priming" or "innate immune memory" (IIM) was mainly phenomenological and the underlying molecular mechanisms remained to be investigated in invertebrates.

To achieve this ambitious goal, we focused our investigations on the snail, Biomphalaria glabrata, for which a specific genotype-dependent IIM was recently reported. This IIM response in B. glabrata is associated with a shift from a cellular immune response after primary infection towards a humoral immune response following the challenge. We demonstrate that this specific IIM was based on the ability for Biomphalaria snails to distinguish different parasite genotypes or parasite stages. We demonstrate the involvement of putative pathogen recognition receptors (PRRs) that varies in qualities and/or quantities following homologous and heterologous immune challenges.

Finally, we investigate the epigenetic and metabolomic supports of IIM and try to reconcile mechanisms with phenomena by focusing our experimental approaches on whole snails and snail immune cells, the hemocytes. We demonstrated that following primary infection, a metabolic and epigenetic reprogramming occurred in snails, resulting in the activation of a strong gene transcriptional process in hemocytes following the challenge. These results prompted us to revisit the artificial dichotomy between innate and memory immunity in invertebrates and led us to ask: how memory of pathogen exposures could be recorded, stored and recalled in invertebrate immune systems?

Immune priming in Tenebrio molitor induced by temperature stress and a fungal pathogen

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Pathogens spreading in insect populations reared at high densities can cause devastating losses in commercial rearing facilities. An improved understanding of insect immunity and interactions between pathogens and insects under their rearing conditions is therefore a crucial aspect of alleviating such disease outbreaks. Insects have a form of innate immune memory called "immune priming" (IP), which protects them from infections when previously exposed to a sub-lethal dose of a pathogen. Abiotic stressors, such as temperature, can also induce IP and increase resistance of insects exposed to pathogens. IP is only beneficial in environments in which the insects are repeatedly exposed to pathogens and having an increased immune response over a prolonged time can have a negative impact on other host traits, such as growth and development. In this study, the IP effects induced by temperature shocks (heat and cold shock) were compared with the IP effects induced by a sub-lethal dose of the entomopathogenic fungus *Metarhizium brunneum* both alone, and in combination to study potential interactions. Pathogen susceptibility and effect on molting of immune primed larvae were assessed after subsequent exposure to a lethal dose of *M. brunneum*. Additionally, immune responses (phenoloxidase activity, hemocyte concentration and antibacterial activity of the hemolymph) of immune primed larvae were measured, alongside larval weight increase, duration until pupation and pupal weight. We discuss how the effect of abiotic and biotic stressors on immunity and development of insects can be considered when manipulating rearing environments to maintain insects that have an increased resistance to pathogens.

First evidence of long-lasting association between viruses and the Black soldier fly, Hermetia illucens

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Black soldier flies (BSF) are regarded as robust insects for their broad diet and are experiencing fast production growth within the insect as feed and food industry. Some BSF farms experience mortality episodes yet very little is known on BSF pathogens, particularly concerning viruses. As traces of contemporary and past viral infections can be mined in transcriptomics and genomic datasets, we undertook a bioinformatic approach to explore publicly available BSF data. Using Virsorter2, CheckV and BLAST on assembled contigs and scaffolds, we uncovered several viral sequences associated with multiple genomic and transcriptomic datasets. In particular, transcriptomic data led to the genome assembly of an uncharacterized virus, that we refer to as "virus T". In parallel, the use of a novel pipeline on three BSF genomes collected in different countries allowed the discovery of multiple candidate endogenous viral element (EVE) sequences. Analysis of the EVEs shared by all three BSF genomes revealed that some EVEs had nearly identical sequences, indicating that their integration in the BSF genome is not recent. Of note, a short sequence that is highly similar to one group of these EVEs was found to be expressed in BSF, suggesting possible antiviral activity. Lastly, sequence comparison revealed that these EVEs are related to, but different from virus T. Altogether, the results suggest that virus T is an exogenous virus producing an active infection, and that related viruses have long been associated with BSFs.

The Consumption and survival rate of *Lilioceris cheni* (Coleoptera:Chrysomelidae) on Air Potato Leaves Exposed to *Cordyceps fumosorosea* (Hyprocreales: Cordycipitaceae)

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Lilioceris cheni Gressitt and Kimoto (Coleoptera: Chrysomelidae) is a biological control agent forthe invasive vine *Dioscorea bulbifera* L. (Dioscoreales: Dioscoreaceae). The beetle is widely effective in the management of the invasive vine in Florida. How biological control agents such as *L. cheni* interact with other insect pest control methods has been a subject of interest to scientists. In the present study, the survival and leaf consumption of *L. cheni* adults exposed to a broad-spectrum entomopathogenic fungus, *Cordyceps fumosorosea* (Wize) (formerly *Isaria fumosorosea*) (Hypocreales: Cordycipitaceae), were investigated via leaf bioassays. *Cordyceps fumosorosea* contains strains that are commercially available in the U.S. and effective biological control agents against agricultural pests such as aphids, psyllids, spider mites, thrips, and whiteflies. The fungus is also reportedly pathogenic against certain chrysomelid species. Adults of *L. cheni* were individually provisioned for the first 24 hours of the experiment with a *D. bulbifera* leaf sprayed with a *C. fumosorosea* suspension of 10^6, 10^5, 10^3, or 10^2 blastospores ml-^{1} or sterile distilled water (control treatment) and with clean (untreated) leaves as needed for the subsequent 20 days. Preliminary results showed the survival and leaf consumption of the L. cheni adults did not differ among treatments and indicated the label or lower rates of *C. fumosorosea* did not significantly increase the mortality of *L. cheni.* Overall, our study demonstrated an inadvertent exposure of *L. cheni* adults to *C. fumosorosea*, such as via spray drift, will unlikely impact the performance of the adult beetles.

Drivers and role of bacterial diversity and composition along the developmental stages of the Black Soldier Fly (*Hermetia illucens*)

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Gut bacterial communities play a key role in a number of essential functions in the different developmental stages of insects, and the Black Soldier Fly or BSF (*Hermetia illucens*) is no exception. Shedding light onto the gut bacterial diversity and composition of BSF is important for its use in waste conversion and animal feed production, being efficient insects at bioconversion processes. The main aim of this project is to characterize the microbial communities along the four developmental stages of BSF: eggs, larva, pupa and adult. We explored whether there were significant differences between the composition and diversity of the four stages and whether these differences were mainly driven by host (vertical transmission) or by diet (horizontal transmission). Finally, we aimed to infer the functions of the main microbial taxa and their role in the development of BSF and its bioconversion abilities. To this purpose, we carry a 16S metabarcoding analysis on sampling comprising 141 BSF specimens, which covered the four main developmental stages of BSF and coming from four different rearing colonies fed with three different diets to. Our results reveal the presence of a core microbiota shared by all four developmental stages as well as significant variations in microbial communities. These variations seem to be mainly driven by the diet composition, in accordance with previous studies. Several bacterial taxa detected in our study seem to play an important role in certain functions carried out in the different life stages of BSF and could be candidates to improve its industrial production for bioconversion processes.

First Record of *Lysinibacillus* sp. From *Varroa destructor* and Potential Bioinsecticide for Honeybee Health

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Beekeeping makes significant contributions to the agricultural economy in Turkey and in the world. It is evaluated that vegetative production can be reduced by 47% in an environment where there are no bees. Colony extinction events, which have recently been observed in different countries and whose cause cannot be explained, are considered as a problem that may affect the biological balance in the future. It is noted that similar problems can be seen in Turkey. In parallel with the importance of beekeeping in Turkey, viral, bacterial, parasitic and fungal diseases seen in honey bees are also very important for the honey production sector. These diseases cause slow development of bees and limitation of their effective production and even extinction of hives Varroa, a parasitic disease, constitutes the biggest problem among these diseases. Varroa is an external parasite that survives and reproduces in the colonies of bees and is highly harmful to beekeeping. Many studies have been conducted on the biology, epidemiology, parasite guest relations, population dynamics, and hormonal, chemical, physical, genetic and biological struggles of Varroa, and protection and control methods against Varroa have been tried to be developed. But there is no biological control method against Varroa. For this purpose, bacterial isolation was performed from Varroa mites. And *Lysinibacillus* sp. was determined. All test were performed to determine for the bacterium (Molecular tests, Biochemical tests, toxin genes, phylogenetic tree, electron microscope). And this is the first isolate from Varroa destructor. This bacterium should be developed for biopesticide.

DISEASES OF BENEFICIAL INVERTEBRATES
POSTERS

A Single Cell RNA sequencing approach to characterize *Biomphalaria glabrata hemocyte* responses in innate immune memory process

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The innate immune system of *B. glabrata* showed memory capacities especially when confronted to *S. mansoni*, the agent of human intestinal schistosomiasis. This innate immune memory (IIM) process is still an extremely complex black box in which the hemocytes, the innate immune cells of *B. glabrata* seem to play a fundamental role. Unfortunately, hemocytes are still poorly defined, three populations have been described based on their morphological characteristics: the hyalinocytes, the granulocytes, and the blast like cells. Herein, we propose to evaluate the complex processes played by hemocyte populations in IIM. Thus, we used Single-Cell RNA sequencing technology to analyse the hemocyte transcriptional response following *S. mansoni* experimental infestation and re-infestation. This approach permitted to discriminate transcriptional infra-populations and trajectories of differentiation. Therefore, we are able to define specific genes related to each hemocyte population and conduct functional enrichment analysis to define hemocyte population sub-functionalization and molecular processes supporting IIM. Finally, using such specific molecular markers, it would be possible to associate transcriptional populations and morphological populations and defined more accurately which and how hemocyte populations could recorded, stored and activated an efficient IIM response in *B. glabrata* snails.

FUNGUS DIVISION

ENTOMOPATHOGENIC FUNGI DIVERSITY 1
VIDEOS

Infection of *Spodoptera frugiperda* with the entomopathogenic fungus *Beauveria bassiana*

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Lepidopteran pests of corn and other grasses cause significant economic losses of more than $1.1 billion annually. The fall armyworm (*Spodoptera frugiperda*) is a damaging pest of corn and sorghum in its native range in North America and is an invasive pest in many regions around the world. Research on pathogenicity of *B. bassiana* towards *S. frugiperda* was conducted with three principal objectives: (1) to test virulence of different isolates of *B. bassiana* against *S. frugiperda*, (2) to identify fungal genes, especially those involved in secondary metabolism, upregulated during insect infection, and (3) to determine genes upregulated during the insect immune response. Insects were infected with spore suspensions (1 x 10^6 spores/mL) of different *B. bassiana* isolates to measure LT50 and time to sporulation. For transcriptomic experiments, larvae of *S. frugiperda* were injected with the sequenced strain of *B. bassiana* (ARSEF 2860) and harvested at 12, 24, and 48 hours post inoculation. RNA was extracted and sequenced to a depth of approximately 10 million reads with 50bp SE Illumina technology. Differential expression analysis identified several genes with potential roles in regulating or mediating the insect immune response. Genes identified as expressed by *B. bassiana* included serine peptidases with known roles in digesting insect cuticle or mediating immune responses in the hemolymph, several heat shock proteins, and hexose transporters. The goal of this research was to identify the most promising isolates of *B. bassiana* for use in biological control against *S. frugiperda* and to characterize potential targets in the insect host.

Diversity of native Hypocrealean fungi infecting the invasive spotted lanternfly in the United States

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In the Eastern United States, populations of the invasive spotted lanternfly, *Lycorma delicatula* (White), can be infected by native fungal entomopathogens, including *Batkoa major* (Thaxt.) Humber and *Beauveria bassiana* (Bals.-Criv.) Vuill. In some locations, localized population collapses have been observed in adult spotted lanternflies caused by these pathogens. In 2018-2020, more than 160 *Beauveria* isolates were collected from spotted lanternfly populations and non-target insects in 19 different field sites in eastern Pennsylvania. Using DNA sequencing, we identified at least 25 different genotypes of *B. bassiana* infecting spotted lanternfly, three of which were abundant and widespread. One isolate of *Beauveria brongniartii* was collected from a vespid (yellow jacket wasp) in one of these field sites but, so far, we have only found *B. bassiana* infecting spotted lanternfly and no other species of *Beauveria*. We recently observed two additional species of Hypocrealean fungi infecting spotted lanternfly at low levels, and these were identified as *Metarhizium pemphigi* and *Hirsutella citriformis*. Therefore, we now know of 4 species of native entomopathogenic fungi infecting this abundant, invasive pest that is spreading in the United States.

Laboratory evaluation of the effectiveness of commercial entomopathogenic strains *Beauveria* and *Metarhizium* for control of the Cornfield Wireworm (Coleoptera: Elateridae)

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The cornfield wireworm, *Melanotus communis* (*Coleoptera: Elateridae*), is an economically important pest of potatoes in the mid-Atlantic with feeding holes left on tubers resulting in reduced marketable yield, and increased susceptibility to phytopathogens. Insecticide use is limited due to environmental policy but mycoinsecticides can provide an alternative option for management of this pest; they have been developed for other wireworm species. A number of insect pathogenic fungi have been registered and commercialized in the U.S. for insect control but there is little data regarding efficacy against wireworms, and almost none for *M. communis* in particular. We conducted a series of soil incorporation and immersion bioassays of the principle commercial *Beauveria* and *Metarhizium* strains (*B. bassiana* GHA, PPRI5339, ANT-03, ERL386, and M. brunneum F52), derived from their formulations, to better understand their potential efficacy for *M. communis*. Larvae were unusually resistant to infection in bioassays that paralleled methods and doses commonly used with other insects. Nevertheless, use of the best of these strains not as soil drenches but on nutritive granules that allow the applied rates to magnify as the fungi grow out and sporulate on the granules in soil, may still provide a useful management approach. The reported work is the first step in evaluating potential of any of these fungi for managing *M. communis*.

ENTOMOPATHOGENIC FUNGI DIVERSITY 1
POSTERS

Management of cotton aphid, *Aphis gossypii* using entomopathogenic *Beauveria bassiana*

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The use of entomopathogenic fungi is an alternative strategy to control agricultural pests, especially having resistance to chemical pesticides. To control cotton aphid, *Aphis gossypii*, 99 isolates of *Beauveria bassiana* were arbitrarily selected for screening. Twelve isolates showed above 80% of virulence on cotton aphids five days after treatment, and subsequent bioassays of these isolates were performed to select two of the isolates with high insecticidal activity against aphids. As part of approach for potential mycoinsecticide development, conidial productivity and thermotolerance of the two *B. bassiana* isolates cultured on ten representative grain-based media were investigated. After 14-days of culturing, the conidia were suspended in a 0.03% siloxane solution for counting conidia, and the harvested conidia were incubated at 45°C and sampled every 30 minutes for two hours to measure the conidial productivity and thermotolerance, respectively. Finally, we selected one isolate showing the highest conidial thermostability and productivity in brown rice (*Orysa sartiva L.*) and foxtail millet (*Setaria italica L.*), respectively. This study suggests that the newly selected *B. bassiana* isolate can be an effective mycoinsecticide against cotton aphids in crop fields.

Keywords: Aphis gossypii, Beauveria bassiana, biocontrol, cotton aphid, entomopathogenic fungi

Susceptibility of *Bruchus rufimanus* Boheman 1833 (Coleoptera: Chrysomelidae) to three entomopathogenic fungi: Limits of conidial suspension sprayings and pledging alternatives in integrated pest management strategy

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Bruchus rufimanus Boheman 1833 is a serious pest of *Vicia faba* L. preventing the valuation of seeds. Limited control methods against this pest suggested few alternatives, out of which entomopathogenic fungi were proposed as promiscuous control levers, but no data precisely describe the optimal method of application on large scale crops. First results from laboratory bioassays on this pest showed a "conidial quantity" dependent lethal response which is not compatible with classical crop spraying. These results are therefore discussed to highlight other application methods, such as attract and infect and endophytic fungi, which could integrate IPM strategy.

Keywords – Biocontrol, Entomopathogenic Fungi, Bruchids, *Bruchus, rufimanus*, IPM

Screening of entomopathogenic fungi for virulence against Emerald Ash Borer eggs

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The emerald ash borer (EAB), *Agrilus planipennis*, is a damaging invasive pest that kills ash trees (*Fraxinus* spp.) and is responsible for huge economic losses across the United States and Canada. As part of an integrated management approach, the use of locally adapted entomopathogenic fungi as biological control agents may help suppress EAB populations and reduce damage caused by the beetles. In a previous study, entomopathogenic fungi associated with EAB galleries were isolated from different geographic areas in Minnesota and identified to species level. The aim of this study was to screen the EAB-associated entomopathogenic fungal collection for pathogenicity and virulence using eggs of EAB. Single eggs were inoculated with a water/Tween 20 fungal spore suspension at a concentration of 1.0 x 10^8 spores/ml and incubated individually in humid chambers at 23 °C, under saturated RH conditions. Larvae hatching was evaluated daily and inoculum recovery was assessed at the end of each trial by surface sterilizing unhatched eggs and plating them on SDA media to verify mycosis. Preliminary results show significant negative effects from strains of *Beauveria bassiana*, *Akanthomyces muscarius*, *Cordyceps farinosa*, *Lecanicillium longisporum* and *Purpureocillium lilacinum* on larval hatching. We report results of initial pathogenicity assays and plans for future field trials.

ENTOMOPATHOGENIC FUNGI DIVERSITY 2
VIDEOS

Antagonistic effects of endophytic *Metarhizium robertsii* in maize against the phytopathogen, *Cochliobolus heterostrophus*

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Fungi in the genus *Metarhizium* (Hypocreales: Clavicipitaceae) are insect-pathogens and endophytes that can benefit their host plant through growth promotion and protection against stresses. *Cochliobolus heterostrophus* is a phytopathogen that causes Southern Corn Leaf Blight in maize that results in significant economic losses in the U.S. We conducted greenhouse and lab-based experiments to determine the effects of endophytic *M. robertsii* on maize defenses and the development of Southern Corn Leaf Blight. We inoculated seeds of maize (*Zea* mays L.) with spores of *M. robertsii* and planted the seeds individually in pots in the greenhouse. At the 3 to 4-leaf stage, we sprayed the youngest true leaf with spores of *C. heterostrophus* and covered the plants with clear plastic to maintain humidity. After 96 h, we evaluated maize for plant height, above-ground biomass, endophytic colonization, disease severity, expression of defense genes and phytohormone content. We recovered *M. robertsii* from 74% of plants grown from inoculated seed. Maize inoculated with *M. robertsii* showed greater plant height and above-ground biomass compared with uninoculated plants. However, height and above-ground biomass of C. heterostrophus-inoculated maize was not different from the uninoculated plants. *M. robertsii* modulated the expression of defense genes and the phytohormone content in maize inoculated with *C. heterostrophus* compared with uninoculated plants. The disease severity of *M. robertsii*- endophytic maize plants was lower than in non-endophyticl plants. These data suggest that endophytic *M. robertsii* can promote maize growth and confer disease resistance, possibly by induced systemic resistance.

Identifying Ecological Relationships among *Beauveria bassiana* and Kudzu Bug, *Megacopta cribraria* – How Does Seasonality and Endophytic Presence of the Entomopathogen Influence Incidence on Kudzu Bug in East Tennessee?

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When kudzu bug, *Megacopta cribraria*, was identified in the United States in 2009 as an invasive species, populations established, increased, and spread throughout the southeastern region at intense rates. Kudzu bug invades urban structures, causing unpleasant scenes for homeowners, and reduces crop yield, such as soybean. Kudzu bug caused about $1,000,000 in soybean yield loss and treatment in Tennessee in 2019. Although initial invasion was rapid throughout the southeastern United States, recent declines in kudzu bug populations have reduced the risk of crop damage to growers. These local and regional declines in populations appear to be influenced by at least two natural enemies, a naturally-occurring entomopathogenic fungus and endophyte, *Beauveria bassiana*, and an accidentally introduced egg parasitoid, *Ooencyrtus nezarae*. *O. nezarae* causes 52% mortality in kudzu bug eggs and *B. bassiana* causes about 90% and 33% mortality in second-generation immature kudzu bugs and adult kudzu bugs, respectively. Endophytic presence of *B. bassiana* in kudzu increases throughout the growing season and, by mid-August, *B. bassiana* colonizes up to 23% of a kudzu vine. Endophytic colonization and entomopathogenic infection increase with seasonal changes. The establishment and success of these natural enemies to cause mortality of kudzu bug will play an important role in driving population dynamics to maintain population declines in the southeastern United States. The purpose of this presentation is to provide information on the distribution, seasonality and impact of *Beauveria bassiana* on kudzu bug, as well as on kudzu, populations in east Tennessee, with implications for the United States.

Effect of *Induratia fengyangensis* volatile compounds on West Indian sweet potato weevil, Euscepes postfasciatus (Fairmaire)

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A fungal endophyte (FEs), *Induratia* spp. (previously well known as *Muscodor* spp.) have ability to produce various bioactive volatile organic compounds (VOCs), some of which are known to toxic or repellent to insect pests. In this study, we isolated and identified *Induratia* spp. from conifer trees in Kyushu district of Japan and evaluated its bio-efficacy against West Indian sweet potato weevil, *Euscepes postfasciatus*, a serious sweet potato pest. For isolating *Induratia* spp., leaves, limbs and flowers of the thirty-three Japanese cedar trees, Cryptomeria japonica, were collected throughout Kyushu district of Japan. A total of 163 FEs were isolated by presence of *I. cinnamomi* as a selection tool and subjected to identification by PCR-RFLP and DNA sequencing of rDNA region. Of the 163 FEs isolates, 4 isolates were identified as *I. fengyangensis*. Production of bioactive VOCs by the four isolates was confirmed by their significant antimicrobial activity against *Escherichia coli* and five plant pathogenic fungi. To evaluate the effects of *I. fengyangensis* VOC discharge, pupal stage of the weevils was fumigated for 7 days until adult emergence. Among these isolates, 2 isolates significantly increased fresh weight of newly emerged male and female adults. Further studies will be conducted on dry weight, which consequently leads to understand fungal VOC effect to the insect pest and a possibility as a biofumigation in storage handling.

Development of seed coatings for Phacelia tanacetifolia with beneficial fungi for plant strengthening and protection against plant parasitic nematodes

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Modern agricultural cropping systems require higher ecological sustainability for example by a balanced crop rotation. *Phacelia tanacetifolia* is used for greening in potato cultivation due to its fine root system and can serve as host plant for various species of plant-parasitic nematodes. However, weather extremes or reduced fertilizer applications are causing problems for growth conditions.

A promising way to strengthen *P. tanacetifolia's*, capability to suppress nematode populations and to enhance the ability to act as a catch crop can be achieved by implementation of potentially endophytic nematophagous fungi such as *Pochonia chlamydosporia*. However, without a tailored formulation its efficacy is greatly reduced especially when incorporated into a commercial seed coating process. Therefore, the overall aim of our research is to develop novel technical seed coatings to increase fungal viability and efficacy.

First, fungal spores produced by submerged cultivation were coated on seeds with novel materials, dried in a rotating drum-dryer and cultivated on agar plates to germinate and grow. Afterwards we were able to detect the fungus endophytically with (q)PCR and light microscopy. We also found that the drying survival of *P. chlamydosporia* was enhanced by coating with potato starch. In Addition, preliminary results indicate that *P. chlamydosporia* can reduce the number of nematodes in roots.

Ongoing experiments will show whether formulation aids can increase survival of the fungus after drying and storage in commercial seed coatings.

ENTOMOPATHOGENIC FUNGI DIVERSITY 2
POSTERS

Multifunctionality of endophytic entomopathogenic fungi: plant growth promotion and *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) control in melon

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There is evidence that some endophytic entomopathogenic ascomycetes (EEA) are multifunction, particularly Beauveria spp. and Metarhizium spp. In addition to their well-known function as biocontrol agents against insect pests, these fungi may act as plant growth promoters by endophytism or rhizosphere competence. This new role depends on the plant species, fungal strain and method of inoculation. This study explores the attributes and ability of *M. brunneum* (EAMa 01/58-Su strain) and *B. bassiana* (EABb 04/01-Tip and EABb 01/33-Su strains) for both biological control of *Spodoptera littoralis* and growth promoting of melon plants by using three inoculation methods. The endophytic colonization of plants and plant growth parameters was determined at 2, 7, 14, 21 and 28 days postinoculation (dpi). The iron siderophore production of each strain was also evaluated, as well as the sublethal effect of the endophytic colonization on *S. littoralis*. The strains EAMa 01/58-Su and EABb 04/01-Tip were able to colonize plant tissues, particularly in foliar spray, with the highest colonization ratios showed at 2 and 14 dpi (26.7 and 30.0% and 10.0 and 11.7%, respectively). However, strain EAMa 01/58-Su promoted better the plant growth in soil applications. Besides, this strain had the highest siderophore production and ability to demineralize Fe. Furthermore, significant effect was observed in the development time of *S. littoralis* larvae that fed on colonized plants, increasing the larval development time up to 50% compared with control ones. These results demonstrate the multifunctionality of EEA to be used for *S. littoralis* control while providing benefits on plant growth.

Characterization and production of *Metarhizium majus* isolated from coconut rhinoceros beetle in Samoa, Philippines and Malaysia

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The coconut rhinoceros beetle (CRB) (*Oryctes rhinoceros*) is one of the most damaging pests of coconut and oil palm trees in tropical Asia and the Pacific Islands. *Metarhizium majus* has proved to be highly pathogenic against CRB, with some strains able to kill 100% of the larvae as early as 14 days after treatment. Several isolates have been obtained from field infected specimens from different countries, which can be developed as biocontrol tools to be integrated in eradication and pest management programs. In the present work, three isolates obtained from Samoa, Philippines and Malaysia were characterized based on morphology, genomic multilocus (ITS, EF-1α and ß-tubulin) phylogeny and growth in different culture media. All isolates produced dark-green, large conidia (12 ± 1 µm length) and clustered in the same clade with *M. majus*. Colonies of all isolates developed faster on Sabouraud agar (SDA) than on Potato Dextrose Agar (PDA) at pH 5, 7 and 9, when incubated at 25°C and growth rate was faster at pH 9 on both culture media. All isolates were able to grow and sporulate at 30°C. Four semi-solid substrates based on cassava, taro, kumara and oat were tested for mass production of conidia as an alternative to the common production on rice. *M. majus* showed limited colonization of the cassava substrate with minimal sporulation, but rapidly developed on oat, taro and kumara substrates producing yields >10^8 conidia/cm^2 and demonstrating the potential of locally produced, economic substrates such as taro and kumara, to replace conventional production on rice.

PHYSIOLOGICAL INTERACTIONS
VIDEOS

Experimental evolution as an approach for increasing virulence in insect pathogenic fungi

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Abstract

Experimental passage has historically been useful for increasing the virulence of baculoviruses but has proven much more challenging for other insect pathogens. Previous studies have commonly adopted naïve approaches in which repeated re-infection of host is expected to lead to increased virulence. Here we adopted a theory-led approach using the fungal pathogen *Akanthomyces muscarius* and an aphid host *Myzus persicae*. We selected for virulence at three scales of competition within host, between host and between population hypothesizing that increased fitness at one or more of these scales would lead to increased virulence. Evolved lines belonging to the within host and between host treatments resulted in significantly increased virulence of the fungus. The observed increase in killing power is based on low single-dose bioassays, further work is in progress to establish changes in LD_{50} and changes to fungal life history traits such as mycelial growth and sporulation in vitro.

Methods

The selection experiment was based on a serial passage experiment approach combined with artificial selection to improve the virulence of the entomopathogenic fungi *Akanthomyces muscarius* towards the peach-potato aphid, *Myzus persicae*. The fungus *A. muscarius*, re-isolated from the whitefly biocontrol product Mycotal (Koppert, NL) was passaged through *M. persicae* for 7 consecutive cycles. Sporulating aphid cadavers were plated on Sabouraud dextrose agar (SDA) and harvested for spores to prepare the inoculum for the next infection cycle. Passage rounds 2, 4 and 6 included a random mutagenesis step to increase underlying genetic variation and counteract genetic bottlenecks caused by passaging. Random mutagenesis was carried out by exposing the inoculum to 60 seconds of UV-C irradiation. A control treatment was passaged through SDA media for 7 consecutive cycles. Replicates of 8, 8 and 4 have been independently evolved for the within host, between host and between population treatments, respectively. The between population treatment had 4 subpopulations within each replicate.

We predict that virulence provides fitness benefits to the fungus at one of three different scales: increased ability to infect hosts (a between population advantage), increased competitive fitness (higher pathogen growth rate) within hosts; increased population size (higher pathogen yield) between hosts. In order to test these hyphoteses, we employed a strict selection regime by propagating the subsequent rounds of infection from: the most successful (highest % mortality in aphids) subpopulation of fungi for the between population treatment; the first aphid that died of fungal infection for the within host treatment; and the first 3-4 aphids that were killed by fungal infection (pooled) for the between host treatment.

Unveiling the Phagocytosis Process in Ixodes *ricinus* Challenged by *Metarhizium robertsii*

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Ticks are less susceptible to entomopathogenic fungi (EPF) than insects. This study aimed to evaluate *Metarhizium robertsii* phagocytosis by *Ixodes ricinus* hemocytes. Ticks were collected and phagocytosis assay (2 and 6 h) was performed. Expression profile of 9 genes related to immune response was performed injecting EPF or PBS into tick females. 2h after infection, RNA was extracted, transcribed and gene expression was performed by qPCR. Gene silencing was performed by dsRNA (GFP-control group). One-Way ANOVA and Tukey's test ($P \leq 0.05$) were used as statistics. Cells that phagocytized EPF reached 40% within 6 h with no difference between 2h and 6h ($P > 0.05$). Regarding the expression profile, 2h after EPF infection, a positive regulation was observed in the expression of 6 genes compared to PBS group. After silencing, the hemocytes showed significant reductions ($P < 0.001$) in phagocytosis indexes when compared to GFP. IrC3-3 was the most representative, whose silencing resulted in a reduction of 60% in the phagocytosis index ($P < 0.001$). In addition, we can infer that our results demonstrate that complement-like molecules are involved in tick phagocytic responses to *M. robertsii*. Blocking host immune response, the pathogen can kill the arthropod much faster. The present study demonstrated for the first time an evaluation of the phagocytosis process in hemocytes of I. ricinus challenged by *M. robertsii*, demonstrating that ticks react differently to fungal infection when compared to insects and that possibly, fungi can also use the process of phagocytosis to escape from tick immune response.

Beauveria bassiana ERL836 and JEF-007 with similar virulence show different gene expression when interacting with cuticles of western flower thrips, *Frankniella occidentalis*

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Insect-killing fungal species, Beauveria bassiana, is as an environment-friendly pest management tool, and many isolates are on the track of industrialization. However, some of *B. bassiana* isolates show similar morphology and virulence against insect pests, and so it is hard to differentiate them. Herein we used two patented isolates, ERL836 and JEF-007, and investigated their virulence against western flower thrips, *Frankliniella occidentalis*, and further analyzed genome structures and transcriptional responses when interacting with cuticles of thrips to see possible differences on the initial step of fungal infection. The two isolates showed no significant differences in fungal growth, conidial production, and virulence against thrips, and they were structurally similar in genome. But, in transcription level, ERL836 appeared to infect thrips easily, while JEF-007 appeared to have more difficulty. In the GO analysis of ERL836 DEGs, the number of up-regulated genes was much larger than that of down-regulated genes, when compared to JEF-007 DEGs (more genes down-regulated). Interestingly, in the enrichment analysis using shared DEGs between two infecting isolates, plasma membrane-mediated transporter activity and fatty acid degradation pathway including cytochrome P450 were more active in infecting ERL836. In summary, the two *B. bassiana* isolates had similar morphology and virulence as well as genome structure, but in transcription level they differently interacted with the cuticle of western flower thrips. This comparative approach using shared DEG analysis could be easily applied to characterize the difference of the two *B. bassiana* isolates, JEF-007 and ERL836.

Secondary metabolites produced by a novel isolate of *Metarhizium robertsii* (CPD006) during mass production

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Metarhizium anisopliae complex species have shown great promise as alternatives to chemical pesticides. Crop Defenders Ltd. is developing an isolate of *Metarhizium robertsii*, CPD006, as a mycoinsecticide for the control of pepper weevil, *Anthonomus eugenii*, in Canada. *Metarhizium anisopliae* complex species produces a wide variety of bioactive metabolites, which contribute to the pathogenesis of the fungus. This study quantified key metabolites, destruxins (DTXs) A, B, cytochalasins C, D, and swainsonine, produced at different time points during mass production and in post-harvest conidia. A negative control containing uninoculated media and positive control isolates for DTXs and swainsonine were included for comparison. Our results showed that compared to the positive control ARSEF 3643, CPD006 generally produced a smaller amount of DTXs A and B during all stages of fermentation. Both DTXs A and B were detected from the extracts of harvested conidia of ARSEF 3643, but not from the conidia of CPD006. A small amount of cytochalasin C was detected in CPD006 during both liquid and solid fermentation, but not in conidia. Cytochalasin D was not detected at any stage. The concentration of swainsonine detected in CPD006 was not significantly different from those detected in the positive control ARSEF 1724. It should be noted that production of secondary metabolites is affected by various variables and synergy and antagonism between compounds must be considered when conducting toxicity assessment. This work will facilitate the registration of a new bio-insecticide in Canada.

PHYSIOLOGICAL INTERACTIONS
POSTERS

Influence of culture medium supplementation on *Metarhizium robertsii* protease production and response to heat stress

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Entomopathogenic fungi can infect arthropods and lead them to death. Nutritional factors may impact the growth of entomopathogenic fungi; for example, different nitrogen sources influence the production of conidia and production of extracellular enzymes. In this study, we optimized the culture medium and solid fermentation period for production of proteases and conidia of *Metarhizium robertsii* ARSEF 2575. We also evaluated the interference of media supplementation with riboflavin and salts on conidial tolerance to heat stress. Fungi were grown on parboiled rice supplemented with different compounds: ammonium nitrate, ammonium chloride, potassium nitrate, sodium nitrate, ammonium sulfate, ammonium phosphate or urea. Two control groups were also tested: positive control (supplemented with sucrose and yeast extract), and negative control (supplemented with sugar). Sodium nitrate showed significant increase in fungal protease production ($p<0.05$) at day 20 of solid fermentation. Then, different concentrations of sodium nitrate and riboflavin were evaluated in a central composite design (CCD). CCD demonstrated that riboflavin (0,001 grams/weight) and sodium nitrate (0,162% grams/weight) influenced positively on both proteolytic activity and conidial production, but without synergism. Supplementation of the culture medium with optimal concentrations of sodium nitrate and riboflavin did not interfere on the conidial germination of *M. robertsii* ARSEF 2575 without exposure to heat stress, but it increased conidial thermotolerance. These results reinforce that tolerance to heat stress is multifactorial, and that conidial thermotolerance can be induced in *M. robertsii* ARSEF 2575 by culture medium supplementation with riboflavin and sodium nitrate.

Kinetic, enzymatic and thermal evaluation of *Metarhizium anisopliae* conidia produced in solid fermentation

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Metarhizium anisopliae CPMa1502 was previously selected for its insecticidal potential against Demotispa neivai Bondar (Coleoptera:Chrysomelidae). To standardize the mass production process of this fungus, laboratory-scale fermentations were carried out in a solid medium based on rice and supplemented with a virulence-inducing nitrogen source and a matrix agent as a thermotolerance inducer. The incubation conditions were 25 ± 2 °C and relative humidity (HR) of 40 ± 10% for 15 days. The dry-weight conidia concentration (conidia/g) was determined from the first day of fermentation using a hemocytometer and conidia moisture content. The concentrations obtained were adjusted to a logistic model to determine the specific growth rate (1.7 -/day) and the doubling time (0.6 day). The maximum conidia production time were 14 days (7-8x109 conidia/g) and productivity of 5x108 conidia/g·día. Chitinase and protease activity was quantified by spectrophotometric methods, observing 0.13 EU proteases/mL and 0.006 EU chitinases/mL. Conidia resistance to thermal and water stress was evaluated, submitted to 45 ± 2 °C, RH of 27.94 ± 0.01% for 5 h. Germination was evaluated before and after the process and the remaining conidia were stored at 30 ± 1 °C for 15 days under vacuum. After this time, germination was determined. It was found that before and after heat treatment the average germination was 98% and 86%, respectively, and after storage, it was 72%. To obtain active principles resistant to thermal stress guarantees a greater tolerance to the operating conditions of drying processes and longer shelf life.

Quantification of filamentous growth of entomopathogenic fungi using spectrophotometry for rapid and high-throughput analysis

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Quantifying growth is the fundamental basis for many studies involving fungi and for many pathogenic fungi related to virulence and pathogenic potential. However, traditional methods for determining growth involving measuring biomass or colony growth area can be time consuming, which limits large scale, multi-factorial studies. Here we develop a method for rapidly measuring fungal growth in small-volume liquid media cultured in 96-well microplates using spectrophotometry. To verify our measurements of growth, change in absorbance over time is compared to dry weight of samples and colony growth area on agar plates. This allows for correlation of absorbance values to quantified biomass and generation of growth curves. We analyse 9 different isolates of *Metarhizium* spp. with this technique for treatments involving different temperature and nutrition gradients. We aim to develop spectrophotometric analysis of liquid cultures in microplates as an effective, reproducible, and simple method for rapidly measuring filamentous fungal growth.

APPLIED ASPECTS
VIDEOS

Evaluation of different *Beauveria bassiana* GHA formulations against overwintering spotted lanternfly (*Lycorma delicatula*) egg masses with various seasonal applications

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The spotted lanternfly [Hemiptera: Fulgoridae: *Lycorma delicatula* (White)] is a phloem-feeding planthopper native to eastern Asia and introduced into Pennsylvania, United States, in 2014, whose wide host range perpetuates its spread and impacts many agricultural, ornamental, and lumber commodities. Since its introduction into the U.S., *L. delicatula* has become partially established in all surrounding states and continues to spread. *Beauveria bassiana* has been shown to be efficacious against the nymphal and adult stages. However, spotted lanternfly overwinters as an egg deposited on many different surfaces, which represents the longest of all its life stages. Management of *L. delicatula* at the egg stage could provide growers and land managers with a practical window to manage the species. Little research has been done on the management of spotted lanternfly egg masses, none with entomopathogenic fungi. We investigated the effects of different commercial formulations of *Beauveria bassiana* strain GHA against overwintering spotted lanternfly egg masses when applied at different seasonal timings. Using this information, we could help develop additional integrated pest management strategies to suppress spotted lanternfly populations.

Virulence of field-collected entomopathogenic fungi to diamondback moth larvae – dose, temperature and host starvation effects

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We evaluated the efficacy of *Beauveria bassiana* and *Metarhizium* species isolated from soil across different vegetation types in south-east Queensland. We measured the pathogenicity of topically inoculated conidia (1 x 10^8/ml) of each isolate to early 3rd instar diamondback moth (DBM) larvae at 20°C. One *B. bassiana* isolate proved to be significantly more virulent than the others, with an LD50 of 3.9 x 10^7conidia/ml, within seven days of inoculation. The effects of inoculation temperature on disease progression were evaluated at 15, 20, 25 and 30°C, after seven days. The mortality rates caused by three *B. bassiana* isolates and four *Metarhizium* spp. were consistently above 70% across the temperature range. Fungal growth in the haemocoel of larvae, disease progression, and sporulation were lowest at 15°C. Above 15°C the isolates responded differentially to temperatures. The impact of 24h of starvation and different temperatures (15, 20, 25 and 30°C) on larvae, given a sublethal topical dose (1 x 10^5/ml) of the most virulent *B. bassiana* isolate was evaluated. The highest mortality occurred in the starved treatment at 30°C. Results show that: (i) different isolates from the field differ significantly from one another in their virulence to DBM, (ii) each fungal pathogen has its own temperature requirements, and (iii) temperature has a more significant effect on the infection and sporulation rate than does starvation of the host. The need to investigate other biological attributes of these entomopathogens, besides virulence, is necessary to understand their ecology.

Keywords: Diamondback moth; *Beauveria*; *Metarhizium*; virulence; temperature; starvation.

Heat stress causes physical damage on the conidial surface of *Metarhizium anisopliae*

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High temperatures may negatively affect both survival and virulence of entomopathogenic fungi. This study assessed the conidial surface of *Metarhizium anisopliae* IP119 exposed to wet-heat stress. Aqueous conidial suspension (Tween 80, 0.01%) of *M. anisopliae* IP119 were exposed to 4 or 8 h at 27 ± 1 °C (controls, unheated) or to 4 h or 8 h at 45 ± 0.5 °C (heated) in a water bath. After each exposure time, 10 µl of each control or heated suspensions were processed for atomic force microscopy (AFM) for physical evaluation of the conidial surface. AFM analysis showed that the integrity and organization of surface proteins (hydrophobins) of heated conidia were altered in relation to unheated conidia, regardless of the exposure time. The surface of heated conidia presented smaller height profiles than the surface of unheated conidia, but the height maps showed small visible changes on the surface wrinkles of heated and unheated conidia. The roughness of unheated conidia was higher than the heated conidia, regardless of the exposure time, and the difference of roughness values between heated and unheated conidia increased proportionally to the time the suspensions were exposed to heat. Heat exposure promotes physical changes on the conidial surface of *M. anisopliae* IP119; these findings reinforce the need for developing efficient formulations to protect the integrity of conidia against high temperatures that may be expected to challenge fungal products in the field.

Fungal tolerance to Congo red, a cell wall integrity stress, as indicator of ecological niche

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Differential sensitivities to the cell wall stress caused by Congo red (CR) have been observed in many fungal species. In this study, the tolerances and sensitivities to CR was studied with an assorted collection of fungal species from three phylogenetic classes Sordariomycetes, Dothideomycetes, and Eurotiomycetes, three orders, and eight families, which grouped into different ecological niches, such as insect pathogens, plant pathogens, saprotrophs, and mycoparasitics. The saprotroph *Aspergillus* niger and the mycoparasite *Trichoderma atroviride* stood out as the most resistant species to cell wall stress caused by CR, followed by the plant pathogenic fungi, a mycoparasite, and other saprotrophs. The insect pathogens had low tolerance to CR. The insect pathogens *Metarhizium acridum* and *Cordyceps fumosorosea* were the most sensitive to CR. In conclusion, Congo red tolerance may be an indicator of ecological niche, accordingly, the tolerances of the fungal isolates to Congo red was closely aligned with their ecology.

The infection mechanism and dynamics of orally administered *Beauveria pseudobassiana* and toxicity of its secondary metabolites in *Anopheles stephensi*

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Entomopathogenic fungi infect insects through oral ingestion of and cuticle contact with conidia. The pathogenicity and infection mechanism of orally ingested conidia in adult mosquitoes are unknown. Therefore, we investigated the infection mechanism and dynamics of orally ingested *Beauveria pseudobassiana* in *Anopheles stephensi*, and also the toxicity of secondary metabolites produced by the fungi. First, a conidia suspension and fungal culture filtrate were orally administered to adult mosquitoes. The LT_{50} values of oral administration for conidial suspension and fungal culture filtrate were 3.9 and 3.4 days, respectively. Second, we dissected and fluorescently stained the alimentary canal of the mosquitoes, and observed the fungal growth under a microscope. Luminescence of fungi was confirmed in the alimentary canal of mosquitoes orally administered with conidia suspension. Conidia germinated and grew in the midgut and hindgut of the alimentary canal. Last, midgut, hindgut, and crop from the fluorescent image of the alimentary canal were separated, their brightness values were calculated and analyzed. The hindgut and crop showed significantly higher brightness than the control group in organ-by-organ comparison. In between organs, the crop was the brightest and significantly higher than the midgut and hindgut. Our results showed that orally administered B. *pseudobassiana* infected from alimentary canal, and caused premature lethality in adult mosquitoes. This provides a new alternative approach to cuticle infection and/or a new strategy to control disease vectors.

APPLIED ASPECTS 2
POSTERS

Making the right decision: Temperature-dependent modelling approach and spatial prediction reveal suitable areas for deployment of two Metarhizium anisopliae isolates for sustainable management of Tuta absoluta

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The tomato leafminer, *Tuta absoluta* is one of the most devastating invasive pests of Solanaceae crops in Africa. We previously demonstrated that *Metarhizium anisopliae* isolates ICIPE 18, ICIPE 20 and ICIPE 665 are potential candidate biopesticides against the adult stage of the pest but adequate strain selection and accurate spatial prediction are fundamental to optimize their efficacy prior to field deployment. This study therefore assessed the thermotolerance, conidial yield and virulence (between 15-35°C) of these candidate isolates. Over 90% of conidia germinated at 20, 25 and 30°C while no germination was recorded at 15°C within 24 hours. Growth of the three isolates occurred at all temperatures, but was slower at 15, 33 and 35°C as compared to 20, 25 and 30°C. Linear and Brière-1 nonlinear models predicted an optimum temperature for mycelial growth at 30°C for all isolates, while the optimum temperature for spore production was at 25°C. Furthermore, ICIPE 18 produced higher amount of spores followed by ICIPE 20 and ICIPE 665. The highest mortality occurred at 30°C for all the three fungal isolates, while the LT_{50} values of ICIPE 18 and ICIPE 20 were significantly lower at 25 and 30°C compared to those of ICIPE 665. Spatial prediction revealed several suitable locations for ICIPE 18 and ICIPE 20 deployment against T. absoluta in Kenya, Tanzania and Uganda. Our findings suggest that ICIPE 18 and ICIPE 20 could be considered as effective candidate biopesticides for *T. absoluta* management base on temperature and location-specific approach.

Keywords: Spore germination, conidial yield, insect-pathogen interaction, thermotolerance, spatial prediction, biological control.

Effect of natural occurrence of Metarhizium spp. on soil arthropod communities in three permanent grassland plots in Switzerland

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Metarhizium spp. are fungal pathogens and natural regulators of arthropods and they are used in biological pest control. In order to better understand and improve natural persistence of *Metarhizium* spp. in soils, a characterization of ecological factors affecting development of soil *Metarhizium* populations is needed. Land-use types and soil physico-chemical parameters have been shown to partially explain variance of *Metarhizium* populations. However, a more complete understanding of the extent of which soil arthropod communities affect the development of these fungi is lacking. The goal of this study was to investigate occurrence and abundance of *Metarhizium* spp. and their effect on soil arthropod communities in three closely located permanent 10x10m grassland plots. For that, two adjacent soil cores were taken from 25 evenly distributed subplots within each of the three plots, one to determine *Metarhizium* spp. abundance, i.e., colony forming units per g soil, and a second to extract arthropods with a Macfadyen extractor. Sampling was repeated four times over one year. A DNA metabarcoding approach targeting subunit-I of the cytochrome-C oxidase, was used to analyze arthropod communities. High spatial and temporal variability was observed for *Metarhizium* abundance and arthropod communities across the three plots and four sampling times. *Metarhizium* spp. abundance significantly explained community structure variation of some specific arthropod groups (e.g. *Lepidocyrtus* spp.). Data provide a first insight into the distribution of soil arthropod communities and their association with Metarhizium spp.. Whether *Metarhizium* abundance actually is driving soil arthropod communities or vice-versa will be the subject of further analyses.

Virulence and natural associations of entomopathogens with adults of the cryptic *Phlyctinus callosus* species complex.

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The banded fruit weevil, *Phlyctinus callosus* (Coleoptera: Curculionidae) is an economically important pest of deciduous fruit and grapevine in the South-Western Cape of South Africa. Recent taxonomic work has uncovered a cryptic species complex, with two significant pest species *P. callosus* and *Phlyctinus xerophilus*. Laboratory bioassays were conducted using *Beauveria bassiana*, *Beauveria pseudobassiana*, *Heterorhabditis indica* and *Steinernema yirgalemense*, to determine differences in susceptibility to entomopathogens between *P. callosus* and *P. xerophilus*. The test arena used was 24-well bioassay plates with an inoculation rate of 200 infective juveniles (IJs)/insect for entomopathogenic nematodes (EPNs) and 10^7 conidia/ml water carrier for entomopathogenic fungi (EPF). Infection was determined after 48 and 96 h incubation for EPN species. An improved method for EPF screening on field collected *Phlyctinus* adults was developed, involving surface sterilization for 2 min in 20% H_20_2 and inoculating insects with entomopathogenic fungi (EPF) using a conidial impregnated filter paper. The insects were incubated on the filter paper in the wells for 18 days and mortality recorded daily. Cadavers were surface sterilized and observed for overt mycosis. Results indicated fungal development in the control group, which were isolated and identified using morphological and molecular techniques. Fungi from the control group was considered to be naturally associated with *Phlyctinus* spp. in agro-ecosystems. Low adult weevil infection was found for all entomopathogen species, with highest mortality being observed on *P. xerophilus* treated with *H. indica*.

Not only a formulation: The effects of Pickering emulsion on the entomopathogenic action of *Metarhizium brunneum*

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Growing global population and environmental concerns necessitate the transition from chemical to eco-friendly pest management. Entomopathogenic fungi (EPF) are a rising candidate for this task due to their ease of growing, broad host range and unique disease process, allowing EPF to infect hosts directly through its cuticle. Yet, EPF's requirement for high humidity negates their integration into conventional agriculture. To mitigate this problem, we formulated *Metarhizium brunneum* conidia in an oil-in-water Pickering emulsion. Conidia in aqueous and emulsion formulations were sprayed on *Ricinus communis* leaves, and *Spodoptera littoralis* larvae were introduced under low or high humidity. The following were examined: conidial dispersion on leaf, larval mortality, conidial acquisition by larvae, effects on larval growth and feeding, and dynamic of disease progression. Emulsion was found to disperse conidia more efficiently and caused two-fold more adhesion of conidia to host cuticle. Mortality from conidia in emulsion was significantly higher than other treatments reaching 86.5% under high humidity. Emulsion was also found to significantly reduce larval growth and feeding, while conferring faster fungal growth in-host. Results suggest that a Pickering emulsion is able to improve physical interaction between the conidia and their surroundings, while weakening the host through a plethora of mechanisms, increasing the chance of an acute infection.

Field evaluation of *Akanthomyces (=Lecanicillium) psalliotae* and development of an Integrated Pest Management strategy against cardamom thrips, *Sciothrips cardamomi*

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Cardamom thrips, *Sciothrips cardamomi* (Thysanoptera: Thripidae) cause huge economic losses to cardamom, *Elettaria cardamomum*, a high-value spice crop, in all its growing regions worldwide. To date, the pest is managed by synthetic pesticides alone, which results in harmful residues in the produce and also pose a serious threat to the environment. In our studies, we evaluated the biocontrol potential of a recently isolated entomopathogenic fungus, *Akanthomyces (=Lecanicillium) psalliotae* under field conditions for two years in two major cardamom growing states, Kerala and Karnataka in India. The trials indicated that four rounds of soil application of the fungus granules reduced the capsule damage by thrips up to 79% compared to control. Moreover, dual application of the fungus as spray and soil application was also found to be effective in reducing the capsule damage by thrips, whereas spray application of the fungus was found to be ineffective. The fungus was found to be compatible with the insecticide, spinosad as well as the fungicide, copper oxychloride. Various integrated pest management (IPM) components involving application of recommended insecticide (quinalphos), reduced risk insecticide (spinosad) and soil application of fungal entomopathogen *(A. psalliotae)* were evaluated for the management of the pest. The trials indicated that initial sanitization of the crop with either of the insecticides followed by three rounds of soil application of the fungus or alternative application of spinosad and the fungus were found to provide an overall protection in capsule damage between 70% and 90%. A quantitative increase in yield was also noticed in soil application treatments. Our findings offer a scope for integrated management of cardamom thrips with reduced risk to the environment integrating the existing cultural method (phytosanitation) along with chemical (spinosad or quinalphos) and biological control *(soil application of A. psalliotae)*. This is the first IPM schedule developed against this major pest of cardamom with biological control as a component.

Post-application persistence and field efficacy of a new strain of *Cordyceps javanica* against the silverleaf whitefly, *Bemisia tabaci* biotype B

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A new strain of *Cordyceps javanica* (Wf GA17) caused widespread epizootics among whitefly populations in cotton fields in southern Georgia in 2017. In previous laboratory tests, *C. javanica* Wf GA17 demonstrated superior temperature tolerance and higher virulence against the silverleaf whitefly, *Bemisia tabaci* (biotype B) compared to the commercial strain *C. fumosorosea* Apopka97. As a follow-up, the post-application persistence and field efficacy against *B. tabaci* of the new strain were compared to *C. fumosorosea* Apopka97. Both fungi were applied in unformulated blastospores alone or mixed with the JMS stylet oil (a horticultural oil). Assessment of leaf samples collected immediately after application indicated that all treatments caused significant mortality and mycosis development in B. tabaci immatures, and there was no difference between the two fungi. However, only *C. javanica* Wf GA17 mixed with JMS oil provided significant control of *B. tabaci* at 7 days after treatment; neither fungi applied alone caused significant insect mortality or reduced the whitefly populations. The lack of persistent control was likely due to the rapid decline of fungal viability, as over 90% blastospores did not survive 24 h after application regardless of fungal strain or addition of oil. Moreover, approximately 80% of the fungal spores were recovered on the upper leaf surface, yet whiteflies reside on the lower leaf surface. Our results indicate areas for improvement in targeting whiteflies with entomopathogenic fungi.

Keywords: entomopathogenic fungus, *Cordyceps javanica*, field persistence, field efficacy, *Bemisia tabaci*

Biopesticide using Entomopathogenic fungi *Beauveria bassiana* Entomopathogenic fungi-mediated management in field

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The red mite, *Dermanyssus gallinae* (Mesostigmata: Dermanyssidae), is one of the most serious pests in chicken farming and causes serious economic losses. Overuse of chemical insecticides has caused pest resistance as well as environmental residual toxicity to chickens and eggs. Therefore, given the warm and humid conditions of chicken farms, alternative application with entomopathogenic *Beauveria bassiana* was investigated to control D. gallinae. Based on the characterization and virulence assays of *B. bassiana* isolates against red mite, a red mite-pathogenic fungal library was constructed. A total of highly virulent six *B. bassiana* isolates were selected from the virulence screening and tested fungal virulence, conidial production, and thermotolerance. JEF-410 considered to be the best candidate for industrialization showed a dose-dependent virulence against red mite nymphs. JEF-410 conidia from millet granule-based mass culture were applied to a commercial chicken farm and it showed a significant suppression of population increase under high humidity conditions. In conclusion, *B. bassiana* JEF-410 having higher control efficacy than cypermethrin could be effectively used to control *D. gallinae* in the fungus-friendly humid chicken farms. Challenge of controlling the red mite using *B. bassiana* in chicken farm, such as formulation and circulation period is possibly improved.

Keywords: *Beauveria bassiana*, *Dermanyssus gallinae*, entomopathogenic fungi, fungal pathogen, red mite

MICROBIAL CONTROL DIVISION

MICROBIAL CONTROL DIVISION
VIDEOS

A Novel Formulation for Baculoviruses Protects Biopesticide from Degradation by Ultraviolet Radiation – Laboratory and Plant Trials with *Spodoptera littoralis* Nucleopolyhedrovirus Confirms Greatly Extended UV Stability

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Baculoviruses, as biological pest control agents for insect pests, have become an established, though currently small, part of the commercial crop protection industry. A significant constraint to their wider uptake by farmers, especially in tropical systems, is their susceptibility to the ultraviolet (UV: 290 – 400 nm) radiation in sunlight, which limits their persistence and efficacy. Developing baculovirus formulations with improved UV stability is seen by many as a major step to expanding the use of baculovirus products.

In this presentation we will describe a novel formulation technology for biopesticides in which the active ingredient (baculovirus) is micro-encapsulated in an ENTOSTAT wax combined with a UV absorbent (titanium dioxide, TiO_2). This encapsulation enables only small amounts of a UV protectant to provide strong protection to the baculovirus occlusion bodies and DNA from degradation by sunlight, but dissolves in the alkaline insect gut to release the virus, which then infects and kills the pest. Trials of the new formulation under simulated sunlight conditions in the laboratory showed that the encapsulation greatly extends the persistence of baculovirus beyond that achieved with existing commercial formulations.

Further trials on cabbage and tomato plants confirmed that this can extend the efficacy of the biopesticide well beyond the few hours of existing virus formulations to many days, potentially increasing the spray interval and/or reducing the need for high application rates.

The novel formulation is relatively cheap to produce, shows no phytotoxicity and has a shelf life comparable with current products. The implications of the ENTOSTAT formulation for lowering the costs and increasing the efficacy of baculovirus insecticides thus expanding the application of baculoviruses to a wider range of pest control situations will be discussed.

Successful selection of a UV-resistant Cryptophlebia leucotreta betabaculovirus for a more persistent biopesticide

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The Cryptophlebia leucotreta granulovirus (CrleGV) has been used commercially for control of the false codling moth, Thaumatotibia leucotreta, on citrus in southern Africa since 2004. Bioassays and field trials with CrleGV showed that despite exposure to UV for several days, there was always some residual activity, potentially indicating the presence of UV-resistant CrleGV-SA in the virus population. The aim of this study was to isolate and characterize this UV resistant CrleGV. Samples of CrleGV were exposed to conditions simulating normal daylight UV, in a Q-SUN Xe-3HC Xenon Test Chamber at various time points ranging from 1 to 72 h. The samples were exposed to UV, propagated in *T. leucotreta* fifth instars and re-exposed to UV. Five exposure and re-exposure cycles were completed. Dose-response bioassays were conducted with *T. leucotreta* 1st instars to determine change in virulence. After 24 h exposure, LC_{50} was 2.89 x 10^8 OBs/ml at cycle 1 and 2.16 x 10^5 OBs/ml at cycle 5. LC50 values after 72 h exposure were 2.11 x 10^9OBs/ml at cycle 1 and 1.73 x 10^6 OBs/ml at cycle 5. This represented a 1338-fold and a 1220-fold difference, respectively, indicating successful isolation of resistant CrleGV-SA. Sequencing of the UV-tolerant samples revealed 14 SNPs after cycle 5, which were thought to be responsible for establishment and maintenance of the UV-tolerance. Work is currently being conducted to determine if these genetic changes and associated UV-tolerance can be maintained after in vivo passage, and whether residual efficacy of the virus is improved in field trials.

LdMNPV baculovirus as a regulator of gypsy moth population dynamics in cork oak forest

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The protection of cork oak forest from the defoliator *Lymantria dispar* (Lepidoptera: Erebidae) is normally based on ecosystem management programs, often involving the application of eco-sustainable microbial products during population outbreaks. However, not many products are commercially available for such applications that in many European countries necessarily requires a specific and temporary authorization, especially in case of aerial treatments. In this context, *Bacillus thuringiensis kurstaki* (Btk) is the most used and successfully employed solution. Another option that was investigated in this multiyear study is the species-specific multicapsid nucleopolyhedrovirus (LdMNPV), an agent of natural epizootics in gypsy moth populations. Experimental trials based on a randomized block design involved a comparison between treated plots (different timing and dose) and untreated check. Experiments were replicated in different forest areas and years, involving bioinsecticidal applications either from ground or by aerial distribution with a helicopter equipped to spray at ultra-low volumes (2 L/ha). A good efficacy of treatments was achieved in all trials, but a higher capacity of pest containment was observed when treatments were done early or at higher doses. While LdMNPV appeared less effective than Btk in the treatment season, effects of baculovirus treatments are expected in the following season as a result of vertical transmission, which foster its use in integrated management programs combining both microbial agents to regulate pest population dynamics.

MICROBIAL CONTROL DIVISION
POSTERS

Developing a sustainable attract and infect strategy for the control of the fall armyworm, *Spodoptera frugiperda*, in Africa

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The fall armyworm (FAW), *Spodoptera frugiperda*, is an economically important pest on staple crops native to the Americas. Its invasion in Africa was first reported in 2016 and has since established throughout the continent, as well as in Asia and Australia. Since pest control with pesticides involves the risk of pesticide resistance development, there is a need for developing more sustainable, safe and environmentally friendly pest control alternatives. Different biological control methods have been successfully used to control several lepidopteran pest species, including viruses. However, viruses are only effective at the early larval stage and resistance development can occur as well. This project aims to combine two approaches: a) sex pheromone lures to monitor adult FAW presence, predict upcoming infestations and time the window of virus-applications, and b) spraying endemically occurring baculoviruses to infect and kill emerging larvae in a way that minimizes the risk of resistance development. Our project focuses on two African regions: Benin (West Africa) and Kenya (East Africa). For accurate monitoring and timing, we are currently identifying whether and to what extent the FAW sex pheromone is region-specific. For the infection part, we are currently identifying and characterizing virulent baculovirus strains that are associated with local FAW populations. Preliminary results suggest region-specific sex pheromone compositions in the West and East African populations of FAW, and the presence of potentially distinct genotypes of baculovirus in screened field samples. The prospects and implications of our first findings are presented in this poster.

Identification of a PGRP-lb gene in *Spodoptera exigua* with antiviral function against *S. exigua multiple nucleopolyhedrovirus* (SeMNPV)

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In insects, imd pathway is involved in antiviral immune responses. Peptidoglycan recognition proteins (PGRPs) can activate imd immune signaling pathway through the recognition of specific peptidoglycans. However, the function of *PGRP-lb* is unclear in *Spodoptera exigua*. Here, we cloned and identified *PGRP-lb* from *S. exigua*, which consists of five exons that encodes a polypeptide of 234 amino acids with a signal peptide and an extracellular PGRP domain. Our results showed that *S. exigua* multiple nucleopolyhedrovirus (SeMNPV) infection induced up-regulation of *PGRP-lb* expression in *S. exigua* cells. Furthermore, up-regulation of PGRP-lb significantly reduced the infection rate of SeMNPV, and inhibited SeMNPV multiplication (reduced the production of polyhedrons and the titer of budding virus). This is the first report of *PGRP-lb* which revealed an antiviral effect in *S. exigua* and provides insights into the function of *PGRP-lb*, a potential anti-SeMNPV mechanism, and a possible target for the controlling of *S. exigua*.

Key words: antiviral; *PGRP-lb*; SeMNPV; *Spodoptera exigua*

Production of *Oryctes* nudivirus (OrNV) through the DSIR 1179 Heteronychus *arator* cell line

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The *Oryctes* nudivirus is regarded as an exemplary "Classical" biocontrol agent for its role in suppression of the coconut rhinoceros beetle (*Oryctes rhinoceros*) (CRB), a severe pest of coconut and oil palms in the tropics. Release of the virus in artificially infected beetles has been sufficient to initiate a cycle of disease in the target CRB population and lead to population suppression. Isolates of the virus are purified after growth in the DSIR 1179 *Heteronychus arator* cell line and can be stored under refrigeration in sterile culture medium for many years without loss in viability. Cryopreservation of the DSIR 1179 cell line has proven difficult with unreliable results, so the cell line has been maintained in continuous culture for >30 years. Despite this the cells remain permissive to the virus and can be used for culture of small amounts of primary inoculum. A seed lot system is used to produce stock and working cultures and maintain the genetic integrity of the virus isolates. The cell line has been used for capture and purification of new isolates of virus, for titration of virus suspensions, and for preparation of standardized inoculants for infection of CRB hosts. We will discuss our efforts in using the cell line to assist with current CRB management efforts being employed within the Pacific region.

MICROBIAL CONTROL WITH PROTEINS
VIDEOS

Granulovirus derived proteins (GVPs) to enhance insecticidal activity of *Serratia entomophila* against grass grub

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Biological control with the bacteria *Serratia entomophila* is limited by slow speed of kill and its restricted host range limited only to one species of New Zealand scarab (*Costelytra giveni*). Granuloviruses are entomopathogenic viruses widely used as safe biocontrol agents, which use a complex enzymatic arsenal to allow virions to enter the insect midgut. Our hypothesis was that enzymes produced by granuloviruses could enhance insecticidal activity of *S. entomophila* by helping the bacterium to penetrate the insect's gut and kill the host faster, with a lower dose. Crude extracts of viral proteins (500 µg/mL) obtained by dissolving occlusion bodies of Spodoptera frugiperda granulovirus SfGV were combined with five concentrations of *S. entomophila* (10^4 to 10^8 CFU/mL) and tested in laboratory bioassays against larvae of *C. giveni*. Larvae significantly ceased feeding (~50% reduction) when exposed to the highest bacteria concentration, same effect that was obtained when viral extract was combined with the five bacteria concentrations. A significant increase in larva mortality was produced by combining the bacterium with the viral extract, with 11days post infection LC_{50} reduced 1000 times. Scanning electron microscopy evidenced changes caused by viral enzymes on the insect peritrophic membrane, the putative mechanism for enhancement. We conclude that the increased activity of the *S. entomophila* resulted from an altered PM permeability which allowed more bacterium cells to reach the midgut epithelium, suggesting that SfGV proteins can be used as additives to improve performance of *S. entomophila*-based biopesticides.

A novel binary pesticidal protein from *Chryseobacterium* arthrosphaerae controls *Diabrotica virgifera virgifera* via a different mode of action to existing commercial proteins

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Due to its high physiological adaptation, the Western Corn Rootworm (WCR) *Diabrotica virgifera virgifera* (LeConte) is considered as one of the most economically important maize pests. To date, commercial insecticidal proteins, used either as biopesticides or expressed in transgenic hybrid maize to manage WCR populations, come from a unique bacterium: *Bacillus thuringiensis*. The efficacy of these proteins has faltered since the appearance of resistant rootworms. In this study, we reported the finding of a new family of binary pesticidal proteins isolated from *Chryseobacterium genius* active against WCR larvae. A drastic weight loss has been observed on WCR resistant strains to Cry3Bb1 and Cry34Ab1/Cry35Ab1 when treated with GDI0005 & GDI0006 recombinant proteins suggesting that these new binary insecticidal proteins may not share the same binding sites as the commercially deployed proteins Cry3B1 and Cry34Ab1/Cry35Ab1 and have probably a new mode of action.

The project Bio-Protect: Target-specific RNA-based bioprotectants for sustainable crop production in a changing climate

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The recently started ERA-NET project BioProtect contributes to reducing the application of chemical pesticides through further development of a bacterial high-quality (hq)-dsRNA production and formulation platform for the delivery of stable and efficient dsRNA formulations for cost-effective crop protection.The application of target-homologous dsRNA triggers host defense through RNA interference. In addition, dsRNA has the potential to also trigger pattern-triggered immunity as a second host defense pathway.

Applying dsRNA as a bioprotectant in agriculture depends on the ability of modulating dsRNA half-life and on means to increase the efficient cellular uptake of the charged dsRNA molecule once applied to the leaf. To solve these problems, we aim to develop customized formulations and application systems to stabilize the dsRNA molecules and to mask the negative charge.

Cationic biodegradable polymers with the ability to neutralize the charges of dsRNA molecules will be presented. Candidate polymers are chitosans, cationic polypeptides, fillers and reswellers. Complex formation, zeta-potential, particle size and size distribution as well as physico-chemical stability on leaf amended with nucleases will be determined by dynamic light scattering combined with REM/TEM and qPCR. A foliar formulation will be developed that also improves penetration into the leaf. In the light of climate change, a major focus will be on determining the efficiency of hq-dsRNA formulations at different dryness-wetness and environmental temperature conditions.

Here, we will present first results on dsRNA production, formulation and efficacy against an aphid, a virus and a phytopathogenic fungus with a focus on the formulation aspects.

Outbreaks of *Rachiplusia nu* (Guenée) in southeastern and southern Brazil are associated with its field resistance to Cry1Ac toxin

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Transgenic soybean varieties expressing Cry1Ac toxin have been extensively cultivated in Brazil since the 2013/2014 growing season, increasing the probability of selecting resistant insects. Outbreaks of *Rachiplusia nu* (Guenée) have been detected in 2020/2021 in southeastern and southern Brazil, together with moderate soybean defoliation. Sampling performed in Bt soybean fields in the 2020/2021, using the beat cloth method and light traps, showed an unusual prevalence of *R. nu*, in some cases ranging from 6 to 8 larvae per linear meter. Very high prevalence of this species was also observed in the light traps, with most of the owlet moths belonging to it. Total number of *R. nu* moths collected in two light traps in 14 sampling nights, between January and February 2021 in Warta, PR State was 8,461 (93% of the collected moths). The maximum number of captures in one night was 3,629 specimens of *R. nu* moths (February 16, 2021) in two light traps. Bioassays performed in January 2012 with field populations of *R. nu* collected in Bento Gonçalves, RS and Ponta Grossa, PR revealed median lethal concentration values ranging from 0.70 to 1.20 µg.mL^{-1} Cry1Ac. In contrast, field populations of R. nu collected in Bt soybean fields during the 2020/2021 growing season were able to grow and reach the adult stage rearing on artificial diets containing 3, 50, and 100 µg.mL^{-1} Cry1Ac as well as on leaves of soybean varieties expressing Cry1Ac.

Two ABC transporters are differentially involved in the toxicity of two Bacillus thuringiensis Cry1 toxins to the invasive crop-pest Spodoptera frugiperda (J. E. Smith)

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The fall armyworm *Spodoptera frugiperda* is a major agricultural pest that has invaded the East Hemisphere since 2016, generating a serious threat to food security worldwide including Africa and Asia. The Cry toxins produced by Bacillus thuringiensis (Bt) have been shown to be effective against this insect pest. In different insect ABC transporters (ABCC2 or ABCC3) have been shown to be involved as receptors of some Cry1 toxins. We analyzed the role of SfABCC2 and SfABCC3 in the toxicity of Cry1Fa and Cry1Ab toxins in this insect pest. Two S. frugiperda SfABCC2 and SfABCC3 knockout strains, coding for potential functional Bt receptors, were created using CRISPR/Cas9 genome editing system. Both knockout strains showed resistance to both Cry1Fa and Cry1Ab toxins compared with the susceptible strain. SfABCC2 knockout strain showed higher resistance to both Cry toxins than SfABCC3 knockout strain, suggesting a major role of SfABCC2 in the mode of action of these Cry toxins. In addition, expression of SfABCC2 and SfABCC3 genes in Trichoplusia ni Hi5 cells also increased the susceptibility to Cry1Ab and Cry1Fa toxins, in agreement with the genome editing results. The double knockout of SfABCC2 and SfABCC3 strain was not viable in contrast to other lepidopteran species. Furthermore, we report here that SfABCC2 or SfABCC3 knockout strains increased their susceptibility to abamectin and spinosad insecticides.

MICROBIAL CONTROL WITH PROTEINS
POSTERS

Insecticidal action of proteins from the crude extract of *Beauveria bassiana* on the Mediterranean fruit fly *Ceratitis capitata*

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Entomopathogenic mitosporic ascomycetes produce secondary metabolites and proteins that may be virulence factors involved in their mode of action. Insecticide proteins as bait may be particularly important in pests which control is still dependent on the synthetic chemical insecticides, such as the Mediterranean fruit fly *Ceratitis capitata*. Since 2003, the Research Group AGR163 "Agricultural Entomology" of the University of Córdoba has provided information on the ability of fungal strains (mainly from *Metarhizium* spp.) to secrete insecticide compounds in liquid medium. In particular, the strain EAMa 01/58-Su from *M. brunneum*. produces an insecticide protein called SIT (secreted insecticidal toxin) active against several pests. However, up to now, this is the first study in which strains from *Beauveria* spp. have produced crude extracts with insecticidal activity by ingestion. Crude extracts dialyzed and adialyzed fractions and crude soluble protein extract (CSPE) of five *Beauveria bassiana* strains have produced mortality values ranged between 3.3% and 100% against *C. capitata* adults, and average survival time values from 3.4 to 5.8 days. The most promising strain was the EABb 10/103-Fil with 56.7% of mortality after the ingestion of the CSPE. It is required a minimum chronic exposition of 72 hours to achieve significant mortality levels. Protease and temperature treatments showed that the insecticidal activity is retained in a highly thermostable proteinaceous fraction. The semi-purification of the CSPE by Rotofor® Cell showed that active compound has a pI between 4.35 and 4.95 and its polypeptide profile in acrylamide gel determined that its molecular weight is closed to 37 kDa.

MICROBIAL CONTROL INTERACTIONS
VIDEOS

Interaction between indigenous entomopathogenic nematodes and the fungus *Metarhizium anisopliae* against late instar false codling moth larvae

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False codling moth (FCM), *Thaumatotibia leucotreta* Meyrick (Lepidoptera: Tortricidae), is an important phytosanitary pest of citrus in South Africa, for which a zero-tolerance approach to its presence in orchards is taken. Several registered control options exist, and are employed against the above-ground life stages of this pest. Options available to manage the soil-dwelling life stages have remained limited. Research concerning the potential of the soil-inhabiting invertebrate pathogens, entomopathogenic nematodes (EPN) and entomopathogenic fungi (EPF), as management tools, is therefore warranted. Individually, the use of several indigenous EPN and EPF have been assessed under both field and laboratory conditions, with promising results. This research aimed to investigate the interaction of three indigenous EPN species in combination with *Metarhizium anisopliae* FCM Ar 23 B3 against late instar FCM larvae. Following dose-response bioassays, laboratory experiments established that when applied simultaneously at either 24, 48, 72 or 96 h post-fungal application, additive interactions dominated for all three EPN species investigated, *Steinernema yirgalemense* 157-C, *S. jeffreyense* J194 and *Heterorhabditis noenieputensis* 158-C. Only one synergistic interaction, although weak, and two antagonistic interactions were observed. As additive interactions have been shown to reach a synergistic level when certain parameters, e.g. timing of application and dose, are optimised, the interaction of these microbes should be further investigated. This will improve our understanding of the role these agents may have as an additional management tool against FCM, when combined.

Mixed pathogen infections and successful transmission: A complex interaction between host pant, timing of infection and pathogen groups

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Mixed pathogen infections are common. Pathogens co-infecting a host may compete for resources or through the host immune system or interfere directly with each other. Similarly, different host plants could influence pathogen co-infection outcomes in terms of host survival, pathogen replication and subsequent pathogen transmission. Interestingly, traits that enable pathogens to be good competitors within the host might not be the same as those that are needed for successful transmission within the host population.

Using the cabbage looper *Trichoplusia ni* larva, its nucleopolyhedrovirus (TnSNPV) and the entomopathogenic fungus, *Beauveria bassiana*, we are exploring how mixed pathogen infections alter replication and pathogen transmission, as well as whether host plant and timing of infection could alter pathogen transmission outcome. We will present data from a semi-field experiment where transmission from a single pathogen species is compared with transmission from insects challenged with two pathogens. Lab data showed that the number of viral transmission stages produced is higher in mixed infections; however, mortality is increased in mixed infections, but the proportion killed by virus declines in the presence of fungus. Thus, the outcome in the field, where host behaviour, pathogen persistence and host plant quality also play important roles, is hard to predict.

Pathogen interactions are complex and these results are particularly important to help understanding how pathogen diversity can be used to improve long-term pest control in the field.

A combined microbial strategy for the biological control of the fall armyworm *Spodoptera frugiperda* in maize

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Spodoptera frugiperda is a polyphagous insect pest native from America and recently reported in Africa, Asia, and Australia. A combined use of entomopathogenic fungi and viruses for *S. frugiperda* control represents an interesting strategy considering their distinct modes of action, that could be complementary. In the present work, the combined application of *S. frugiperda* nucleopolyhedrovirus SfMNPV and *Metarhizium rileyi* was evaluated under greenhouse and field conditions in maize crops. Five treatments combining both pathogens in different virus:fungus ratios that reached a total concentration virus + fungus in 1 x 10^7 propagules/mL were sprayed onto young maize plants grown in pots. Plants were infested with second instar larvae, which were recovered after 5 days. The treatment using both pathogens in ratio 50%:50% caused the highest mortality with 60% on day 5 and 95% on day 9 post application. Signs of combined infection in larvae were evident, but virus symptoms prevailed. Then, individual, simultaneous, and sequential applications of both biocontrol agents were evaluated (2 applications). After the first application, the lowest recent damage was observed in all treatments where the virus was applied. Two days after the second application, all treatments presented significantly less damage than the control, and no damage was detected in the treatment where only virus was applied. Finally, three application frequencies (at 30% damage level, weekly and biweekly) were evaluated under field conditions using the combination virus:fungus (50%:50%). All treatments significantly reduced the recent damage confirming the potential of integrating both pathogens for efficiently control *S. frugiperda*.

Synergism between a baculovirus and an insect growth regulator?

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Most baculoviruses possess an ecdysteroid glycosyl transferase (egt) gene, which overcomes larval moulting, resulting in increased feeding, slower rate of kill and increased virus production, thus in favour of the virus. Unfortunately, the delayed mortality and continued feeding is drawback for a biopesticide. Consequently, the egt gene can be deleted to restore moulting and improve the speed of kill. Ecdysone agonist (moult accelerator) insect growth regulators (IGR) work on a similar principle. The IGR within the product binds to the ecdysone receptor complex in the larva and mimics moulting, resulting in a faster death of the larva. The aim of this study was to determine whether there would be a synergistic effect between Cryptophlebia leucotreta granulovirus (CrleGV) and an IGR. Synergism was examined using surface dose bioassays with neonate *Thaumatotibia leucotreta* larvae. Bioassays were conducted by combining a serial dilution of CrleGV with a constant concentration of the IGR. Results were analysed using the Tammes-Bakuniak graphical method. Variable results were recorded, however majority of treatments indicated an antagonistic relationship between the treatments. Time response bioassays were then conducted to determine if a synergistic effect could be recorded in improved speed of kill. Although the IGR still had the fastest speed of kill compared to CrleGV alone and in combination with the IGR, the IGR in combination with CrleGV showed an improved speed of kill compared to CrleGV alone. Further research is being focused on identifying other IGRs and natural insecticides, including microbials that could potentially result in synergism with CrleGV.

Innovative formulations for biological plant protection in horticulture

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For several years, insect pests have become a serious economic problem in the European blueberry cultivation. Above ground *Drosophila suzukii*, ovipositing in ripening fruit, is particularly damaging. Below ground, larvae of the black vine weevil and grubs feed on the roots leading to the loss of entire blueberry rows. The ban of many chemical insecticides and the market's desire for residue-free products increases the demand for suitable control solutions. However, effective biological preparations are often expensive, and suffer from a slow killing speed and unfortunately, inconsistent efficacy.

Recently, new virus strains specifically against *D. suzukii* have been isolated that are promising candidates for biological pest control. However, the viruses' infectivity rapidly declines under environmental influences. Besides, an adapted viral formulation and application techniques are needed to ensure high infectivity of the virus.

Below ground, the plant treatment with an entomopathogenic fungi like *Metarhizium brunneum* is promising. In line with the viruses, the effectiveness is also drastically reduced if the fungus is applied unformulated.

To solve these problems, the overall aim of the joint project "HOPE" is the above- and below-ground protection of blueberries by effective spray application with a novel virus formulation and an innovative soil formulation based on the attract-and-kill principle with an entomopathogenic fungus as kill component.

First results on screening for suitable viruses and the development of new cultivation and formulation techniques as well as initial approaches on the development of formulations for entomopathogenic fungi will be presented.

Less is More; Improved Control of *Trialeurodes vaporariorum* by Co-Application of an Entomopathogenic Fungus and an Insect Growth Regulator.

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Greenhouse whitefly (*Trialeurodes vaporariorum*) are a major global pest, causing direct damage to >300 plant species and transmitting viral plant diseases. Management of *T. vaporariorum* using chemical pesticides is difficult because of widespread pesticide resistance. There is an increasing drive to use integrated pest control strategies, including limited chemical application and increased use of sustainable alternatives; Entomopathogenic fungi (EPF) may potentially be used as one such control method. Co-application of a chemical insecticide with an EPF can improve pest control, resistance management and increase the range of environmental conditions over which control is effective. However, co-application has the potential to result in both positive and/or negative interactions. Positive interactions result in synergism which can be exploited for improved pest control, whilst negative interactions cause antagonism which can be mitigated for, if all interactions are understood. In this study, interactions between an EPF (*Cordyceps farinosa*) and the chemical insecticide Oberon® (Active ingredient: spiromesifen) for control of *T.vaporariorum* were evaluated in laboratory bioassays. Here we outline the use of an ecotoxicological 'MIXTox' model to describe the range of interactions between the fungus and insecticide, identifying both synergism and antagonism. When the toxicity of the mixture was mostly caused by spiromesifen, there were negative interactions. However, higher mortality of *T.vaporariorum* was observed than expected with high concentrations of EPF combined with low concentrations of spiromesifen. By improving our understanding of the ecological interactions occurring between biological and chemical control in integrated pest management strategies, optimised approaches can be developed to control crop pests.

Suppressive soil communities as potential insect pest control tools.

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Disease suppressive soils from the Payerne region in Switzerland support the abundance and activity of beneficial microorganisms, specifically *Pseudomonas* bacteria, due to their physicochemical characteristics. These soils can significantly reduce the plant damage caused by the black root rot fungus *Thielaviopsis basicola*. However, it is unknown to which extent and how their microbial communities may affect pest insects which cause great yearly crop losses worldwide.

We aimed to assess the leaf damage and mortality of larvae of the cereal leaf beetle *Oulema melanopus* after herbivory on wheat growing in suppressive and non-suppressive soils. Our first results indicate that the resident microbiota of the suppressive soils reduces *O. melanopus* survival on wheat.

Additionally, we assessed the survival of the biocontrol agent Pseudomonas protegens CHA0 tagged with GFP when it is introduced into extracts of a suppressive soil with and without resident microbial community. *P. protegens* CHA0 was able to grow in the extract that contained the resident microbiota but worse when alone. Finally, as a proof of concept for potential applications, we observed the survival of this strain when co-inoculated with a close relative into wheat rhizosphere and injected into the hemolymph of *Galleria mellonella* larvae. We show that closely related *Pseudomonas* do not necessarily compete when colonizing plant-roots but they antagonize upon insect infection which could be detrimental in possible future pest control applications.

Our study provides insights for a better understanding of the importance of the agricultural soils microbiota to prevent insect invasions and to sustain introduced biocontrol agents.

MICROBIAL CONTROL INTERACTIONS
POSTERS

Effect of interactions among nucleopolyhedrovirus and *Metarhizium rileyi* on the mortality of *Spodoptera frugiperda* larvae under laboratory conditions

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Combining biocontrol agents (BCAs) with different mechanisms of action is a strategy to improve its effectiveness. Worldwide, *Spodoptera frugiperda* (Lepidoptera: Noctuidae) is a highly polyphagous, harmful and invasive species that feeds on more than 76 families of host plants, including essential crops such as maize, sorghum, rice, cotton, alfalfa, and forage grasses. However, *S. frugiperda* exhibits several natural enemies such as entomopathogenic bacteria, viruses, and fungi. In the present work, the performance of mixed infections using a *S. frugiperda* nucleopolyhedrovirus SfMNPV and *Metarhizium* rileyi was assessed on *S. frugiperda* larvae under laboratory conditions. Second instar larvae were inoculated via per os with the virus and via topical application with the fungus. The treatments consisted in each pathogen applied alone or combined using different SfMNPV:M. rilery ratios (high, medium and low). When the highest concentration of SfMNPV or *M. rileyi* were combined with the lowest concentration of the other BCA, infection symptoms exhibited by dead larvae corresponded exclusively to those produced by the pathogen in higher concentration and mortality percentages did not differ from those obtained with these pathogens applied alone. However, mixing the medium concentration of each pathogen, significantly increased the mortality in comparison with the pathogens applied individually, suggesting a synergistic effect. Approximately 25% of the dead larvae showed symptoms of viral (soft integument) and fungal (sporulation) infection demonstrating a dual infection. Results suggested that the integration of both BCAs could be a reliable mean of improving efficacy and reducing the variability of biological control against the fall armyworm.

MICROBIAL CONTROL WITH FUNGI
VIDEOS

Chitin amended media: A solution for improved entomopathogenic fungi against codling moth

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As a resource of biodiversity, entomopathogenic fungi (EPF) can function as natural antagonists of pest species. They can be found in different climatic zones and agroecosystems. The main goal of the bilateral project "Bio-Entosource" is to study the biodiversity of EPF in order to develop new conceptual strategies for a biological pesticide for sustainable use in agriculture and forestry. The scientific cooperation between Germany and Brazil serves to validate the optimization of EPF in interaction with harmful insects in apple, soybean and eucalyptus in two different eco climatic zones.

In Germany, more than 40 EPF have been isolated from organic apple orchard soil samples by using insect Bait-method. Consequently, standardized pathogenicity assay and agar-chitin plate assay has been carried out to select potential EPF. Biological efficiency of one potential EPF *Cordyceps fumosorosea* (JKI-BI-1496) is now being evaluated through several experiments of production and formulation with different media compositions consisting of chitin and it's derivatives to optimize the potential efficacy against *Cydia pomonella*. Among all the media compositions, one medium with 1% colloidal chitin showed the highest efficacy with more than 65% mortality rate. This composition also resulted in higher biomass content and faster germination than other chitin derivatives.

Results on the fungal growth in this media composition in a 4 litre fermenter with defined oxygen concentration, pH and temperature, will be discussed with focus on submerged spore fermentation. All these findings can be served as a new path that stabilizes EPF in order to ensure its effectiveness against *Cydia pomonella*.

Impact of tannins from bioactive plants on the growth and spore production of the biocontrol fungus Duddingtonia flagrans.

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Fungi have been both beneficial and detrimental to many different industries and systems from food production and molecular breakdown to parasitism and health. One such organism that has seen an increased interest, for parasitic nematode control is *Duddingtonia flagrans*. This has come at a time when interest in other alternatives such as plant secondary metabolites (PSM) is also increasing. The potential overlap and interaction between traditionally beneficial and non target organisms such as D. flagrans and PSM must be investigated. The current study aims to establish the basic nature of this interaction and *D. flagrans* tolerance for PSM specifically tannins at different concentrations, agar and temperature. This was achieved through growth plates and spore counts. The growth and spore production of *D. flagrans* where both found to be negatively impacted by the presence of tannins. The current study establishes a direct negative interaction between tannins from different sources and *D. flagrans*. This negative interaction was also found to be temperature dependent. Further research should investigate the impact of this interaction in the field and on the saprophytic stage to see if the interaction is consistent across feeding types.

What is the effect of geographic and temporal separation of the Common cockchafer on the population structure of its main fungal pathogen?

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The fungal genus *Beauveria* (Bals.) Vuill. (Ascomycota: Hypocreales) is globally distributed and comprises twelve insect pathogenic species, including *B. pseudobassiana* and *B. brongniartii*. While *B. pseudobassiana* has a broad host spectrum, *B. brongniartii* specifically infects Melolontha melolontha Linnaeus 1758 (Coleoptera: Scarabaeidae), a widespread pest throughout Europe. *B. brongniartii* is recognized as the most important natural antagonist of *M. melolontha*, and two strains are commercially available biological control agents. It has been hypothesized that adaptation or co-evolution of *M. melolontha* and *B. brongniartii* may have led to changes in virulence and genetic population structure of *B. brongniartii* at the geographical level. No detailed population genetic analyses have been performed on *B. brongniartii* so far, and it is unknown whether local adaptation and selection processes affect the population structure of the fungus. The goal of this study was to investigate and compare the genetic diversity of geographically distinct populations of *B. brongniartii*. A collection of 697 Beauveria spp. isolates from infected *M. melolontha* adults from 45 locations across Europe was established. The isolates were genetically analyzed using the ddRADseq approach and 616 high-quality genome-wide SNP markers were detected. Phylogenetic analysis identified 411 isolates as B. pseudobassiana, indicating that this species and not *B. brongniartii* may be the most prevalent antagonist of *M. melolontha* adults. Further analyses will allow investigation of the genetic population structures of both *Beauveria* spp. and provide information on their distribution and diversity, an important step to further improve *M. melolontha* biological control strategies.

A mycelial–conidial formulation of a silkworm-safe isolate of Hirsutella thompsonii to control Polyphagotarsonemus latus in mulberry

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The broad mite, *Polyphagotarsonemus* latus, is threatening to become a persistent and intractable pest of mulberry, *Morus alba*, in southern India. This pest has firmly established itself in Karnataka, Andhra Pradesh and Tamil Nadu states where sericulture is a major occupation. Despite the easy access to acaricides such as fenazaquin, propargite and sulphur, farmers are in favour of safe alternatives because of the residual toxicity of these chemicals to the silkworm, *Bombyx mori*. The acaropathogenic fungus *Hirsutella thompsonii* [ICAR–NBAIR–MF(Ag)66], in the form of a mycelial–conidial formulation, consistently gave 90% or more control of the broad mite in a series of field trials conducted from August 2019 to January 2021 in Ramanagara district of Karnataka. A 1% concentration of the formulation, applied as a stand-alone treatment thrice at weekly intervals, was comparable to or better than the recommended acaricides in terms of reducing the mite population. As regards safety, *H. thompsonii* could not infect the silkworm in laboratory studies. Likewise, the silkworms that fed on mulberry leaves from fungus-sprayed fields developed healthily and produced normal cocoons. The challenge at hand is to make the formulation of this effective isolate of *H. thompsonii* available to the needy farmers.

MICROBIAL CONTROL WITH FUNGI
POSTERS

The ingestion of Metarhizium-colonized plants produces direct and indirect effects on the cotton leafworm Spodoptera littoralis

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The present work reveals the occurrence of intermediate *S. littoralis* larval mortality levels related to the foliar application of the entomopathogenic endophytic fungus *Metarhizium brunneum*. The mortality rates of larvae fed on *Metarhizium*-colonized melon leaves were 45.0% and 87.5%, and the average survival times were 6.6 and 3.1 days in experiments performed with melon discs and with the whole plant, respectively. Notably, these mortality levels were not associated with fungal outgrowth and were not caspase 1, 3-7 and 8 mediated. This work also shows the existence of significant sublethal effects of food consumption by *Spodoptera littoralis* larvae challenged by the fungal endophyte when feeding on colonized leaves. In this regard, in experiments performed in planta, plant damage increased larval mortality in both fungally challenged and control larvae. There was also a meaningful effect of exposure to *Metarhizium*-colonized plants in female fecundity and egg fertility of the adults emerging from pupae developing from surviving larvae. Experiments performed with foliar discs showed a non-realistic situation due to the prior damage caused to the plant. Hence, the present work presents new findings revealing the high potential of endophytic entomopathogenic fungi to improve the outcome of foliar applications against chewing insects in the short, mid- and long term, by the reduction of the reproductive potential of surviving adults.

Tick cuticle lipids may limit infection by entomopathogenic fungi

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Chemical composition of tick cuticle acts as a barrier to pathogens. Here, we tested the toxicity of total cuticle extracts from four ixodid tick species predominant in Brazil to conidia of *Metarhizium robertsii* IP 146 and *Beauveria bassiana* s.l. IP 361. Cuticular lipids were extracted using hexane, and fungal conidia were scraped from culture plates and suspended in tick cuticle extracts and incubated overnight at room temperature. In the control groups, conidia of IP 146 or IP 361 were also suspended in hexane for the same period. After exposure to the cuticle extracts or hexane (control), the conidia were re-suspended in PBS solution and propidium iodide. The viability of conidia was examined in a FACSCanto II flow cytometer by the acquisition of 10,000 events, and the data were analysed using the FACSDiva software. The extracts from Amblyomma sculptum caused apoptosis to 81% of *M. robertsii* and 36% of *B. bassiana* treated conidia, whereas extracts from Dermacentor nitens caused apoptosis of 64% and 66% to M. robertsii and B. bassiana, respectively. Cuticular extracts from *Rhipicephalus microplus* or *Rhipicephalus sanguineus* caused low (<8%) conidial apoptosis on both isolates, IP146 or IP361, which did not differ from the control group. This study indicates a natural tolerance of *A. sculptum* and *D. nitens* to fungal infection in comparison to *R. microplus* and *R. sanguineus*, although the mechanisms in which they are used for host defence remains uncertain.

Conidial production from granules of Metarhizium humberi microsclerotia on soil samples

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Metarhizium humberi produces structures named microsclerotia which are promising to control soil-dwelling arthropod pests. This study compared the efficacy of microsclerotia of *M. humberi* IP46, formulated as granules or pellets, to produce conidia on soil samples. Microsclerotia were produced by liquid fermentation in a medium with C:N 30:1 ratio, at 250 rpm, for 4 days. The granules were prepared using a 1:1 biomass:excipient ratio and water as liquid binder. Granulation was performed using a 0.5 mm sieve. Pellets were prepared by extrusion-spheronization at the same biomass:excipient ratio. Granules and pellets size distribution was determined by sieving. Soil samples were collected from open grounds or vegetated regions, autoclaved, and distributed in Petri dishes. Granules or pellets were distributed over the soil samples and incubated for 10 days. Then, the soil in each dish was suspended in 0.01% Tween 80 and an aliquot inoculated onto CTC selective medium for entomopathogenic fungi; the colonies and the conidia produced were counted on the fourth day of cultivation. Granules size distribution ranged from 0.1 to 0.7 mm, whereas the pellets size ranged between 0.4 and 0.8 mm. The conidial production from pellets was significantly lower compared to the production from granules, which was approximately $3x10^8$ conidia g^{-1}, with viability higher than 90%. Conidial yield from granules or pellets applied to the soil collected from vegetated regions was higher than the production from those applied to the open ground soils. Formulated microsclerotia have biotechnological potential for controlling arthropod pests that inhabit the soil.

Influence of abiotic factors on the persistence and viability of microsclerotia produced by the entomopathogenic fungus Metarhizium spp. (Hypocreales: Clavicipitaceae)

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Some species of the entomopathogenic fungus *Metarhizium* may produce microsclerotia (MS) as resistance spores to counteract adverse nutritional or environmental conditions. These propagules can be used as active material of a commercial biopesticide for the control of soil-dwelling stages of some important geophilic insect pests. In this study, the MS production and quality of *Metarhizium brunneum* EAMb 09/01-Su and EAMa 01/58-Su and *M. robertsii* EAMa 01/158-Su strain have been evaluated. The three strains were able to produce MS in liquid fermentations with values of produced conidia per MS ranging between 2 x 10^{10} and 4.73 x 10^{10} conidia per gram of MS. Soil texture had a significant effect on EAMa 01/58-Su MS germination and subsequent production of conidia being higher in sandy soils than in clayey soils. The best combination of temperature and soil humidity for the germination of the EAMa 01/58-Su MS was 22.7°C and 7.28%, respectively. Furthermore, the exposure of these MS to UV-B during 4, 8, 24 and 48 hours did not significantly affect their capacity to germinate and produce conidia, demonstrating their photo-resistance. Finally, storage temperature was evaluated over a year, the MS showed longer shelf life when stored at lower temperature, being the -80 oC the one that provided the highest viability of the MS with 2 x 10^9 conidia per gram, after 8 months of storage, while at 25°C its viability decreased to 1 x 10^7 conidia per gram in first four-months of storage.

Digging into the past: *Metarhizium brunneum* as control agent against the sugar beet weevil *(Asproparthenis punctiventris)*

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The sugar-beet weevil (*Asproparthenis punctiventris*, Germ.) is one of the main pests in sugar beet cultivation and causes major damage in Austria. Although biological control of the pest with the entomopathogenic fungus *Metarhizium* spp. was already mentioned and tested in the late 19th century, no further studies have been conducted since then. Therefore, a three-year study in Lower Austria has been initiated to test the effectiveness of *M. brunneum* in preventing larval development and the control of the beetles by using two types of *Metarhizium* formulations (i.e. GranMet GR and GranMet WP). GranMet-GR (active ingredient *M. brunneum* BIPESCO 5) was applied in eleven arable fields with a concentration of 100 kg ha^{-1}. Fields were sampled in spring and autumn to assess Metarhizium abundance and control efficacy. The desired fungal density of 5,000 *M. brunneum* CFU g^{-1} soil (dry weight) and more was reached and even exceeded after the first application of the GranMet-GR in the soil, on its own or in combination with the liquid formulation (1 x 10^{12} spores ha^{-1}). At all the sites, a strikingly high abundance of indigenous *Metarhizium* was found. Genotyping confirmed successful establishment of the applied strain BIPESCO 5 in the treated fields. The fungus was able to persist on treated sugar beet leaves for more than three weeks. In addition, more than 50% of the collected beetles were infected with the applied strain after spray application. Additional trials will be performed in 2021 to confirm the applicability of the *Metarhizium* based sugar beet weevil control.

MICROBIAL DIVISION

INSECT MICROSPORIDIA: HOST PATHOLOGY AND DISEASES CONTROL
VIDEOS

Expression of scFv-fragments against *Vairimorpha (Nosema) ceranae* hexokinase and ATP/ADP carriers suppress microsporidia intracellular development in Sf9 insect cells.

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A prohibition on the use of fumagillin for bee treatment and shutdown of its production by Medivet Pharmaceuticals company in 2018 demand the search of novel strategies to suppress micropsporidia infections in domesticated insects. Along with RNA interference, the development of microsporidia in insect cells should be effectively suppressed by the expression of single-chain antibodies (scFv fragments) against functionally important parasite proteins. An important role in relations of microsporidia with infected cell play non-mitochondrial, plastidic-bacterial ATP/ADP- translocases importing host-derived ATP, and the enzyme hexokinase which secretion by parasites is followed by accumulating in host nuclei. In this study, we constructed an immune scFv library against *Vairimorpha (Nosema) ceranae* hexokinase overexpressed in E. coli and isolated by IMAC as an active enzyme. Another library was constructed against outer loops of four V. ceranae ATP/ADP-transporters fussed in one chimeric protein. Expression of selected by phage display scFv genes in Sf9 cells was followed by their infection with V. ceranae spores. RT-PCR analysis of parasite beta-tubulin expression in infected cultures demonstrated that some recombinant antibodies effectively suppressed microsporidia intracellular development. This confirms an important role of ATP/ADP carriers and hexokinase in the relationships between microsporidia and infected host cells. Further expression of such scFv-fragments in insect cells may increase their resistance to microsporidial infections. This work was supported by the Russian Science Foundation (RSF 18-16-00054).

The gut parasite *Nosema ceranae* impairs olfactory learning in bumblebees

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Cognition, as associative learning and memory, is essential for pollinators to navigate and forage to feed their brood. Stressors may affect these abilities, thus compromising pollinators' fitness and survival. The microsporidia *Nosema ceranae* is a parasite of honey bees that is nowadays spread worldwide and it is also found in a wide range of wild bee species, as bumblebees or solitary bees. How *N. ceranae* does affect bee cognition is, however, little explored. Here, we used the bumblebee Bombus terrestris, as a model species of wild pollinators, to investigate whether *N. ceranae* affects different forms of learning by applying the classical conditioning protocol of the proboscis extension reflex (PER). Seven days after feeding the parasite bumblebees were less able to associate an odour with a reward, discriminate between odours and learn a new association, indicating that *N. ceranae* impaired learning of bumblebees. However, no effect on memory were observed. We discuss the potential mechanisms underlying this sublethal effect of *N. ceranae* exposure on bumblebee cognition, and the consequences for populations of wild pollinators in general.

Nosema pyrausta as natural mortality factor of *Ostrinia* moths

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Nosema pyrausta is among main mortality factors of *Ostrinia* moths. First described in France, it was reported in several European countries and observed in European part of Russia at prevalence levels of 3-17% in 2005-2010 and 0-16% in 2011-2016. *Nosema*-like disporoblastic sporogony prevailed but a single case of additional octosporoblastic sporogony was also detected. Under laboratory conditions, *N. pyrausta* demonstrated similar virulence indices in *Ostrinia nubilalis, Ostrinia scapulalis* and *Ostrinia furnacalis*. During three laboratory generations, the pathogen showed stochastic fluctuations of the prevalence levels in the range of 29 and 92%. Vertical transmission of *N. pyrausta* is achieved by infecting egg cytoplasm with prespore stages of the parasite (true transovarial infection), as inferred from cryosection staining, amplicon sequencing and reverse transcription PCR. Optimal thermal regime for the host development was also optimal for the parasite's spore production in insect pupae, while elevated temperature decreased the spore yield, and lowered temperature provoked early larval death. A diet modification allowed for increasing the spore yield.

Ribosomal DNA sequencing confirmed that the specimens from France and Russia are identical, while an isolate from North America, referred to as *Nosema cf pyrausta*, displays a closely related yet distinct haplotype. The effects of the latter parasite on density dynamics of American populations of the maize pest *Ostrinia nubilalis* is well documented. Yet, as it possibly represents a different species, these data should be interpreted with caution when ecological interactions of microsporidia and *Ostrinia* in Europe are considered. Supported by RSF, project # 20-66-46009.

The microsporidium *Nosema pyrausta* in the beet webworm, *Loxostege sticticalis*

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Nosema pyrausta (Np) is an important mortality factor of the European corn borer *Ostrinia nubilalis*. The present study was aimed at *Np* virulence testing to the beet webworm *Loxostege sticticalis (Ls)*. This agricultural pest was highly vulnerable to *Np*. The parasite's spores were located in salivary glands, adipose tissue and Malpighian tubules of the infected specimens. In transovarially infected insects maintained for three laboratory generations (F1-F3), Ls fitness indices were lower than in control and moth emergence and fertility decreased most prominently. The transovarial infection was most detrimental for the female egg-laying ability, resulting in zero fertility in F3. Further, virulence of *Np* to *Ls* was compared between the isolates propagated in alimentary infected Ostrinia spp. and Ls, as well as transovarially infected *Ls*. As a result, mean survival time (LT_{50}) constituted about 10 days when the insects were exposed to the spores isolated from Ostrinia spp. Meanwhile, Ls displayed LT_{50} of about 8 days when infected with *Np* from either alimentary or transovarially infected *Ls*. These values were significantly different at $p<0.05$. Thus, when propagated in *Ls*, the microsporidium tended to increase its virulence to this host. It can be concluded that Np is a promising agent against the beet webworm which is a notorious polyphagous pest in Eurasia. The parasite's ability to infect this host at low dosages and transmit vertically should guarantee effective establishment and spreading within the beet webworm populations. The research is supported by RSCF grant # 20-66-46009.

Nosema bombycis suppresses host cell apoptosis via Nbserpin14 inhibiting the host Caspase protease BmICE activity

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Nosema bombycis is the pathogen of the silkworm that belongs to the microsporidia, which causes Pebrine disease and brings huge economic losses to the sericulture industry. Apoptosis not only is important for organismal homeostasis but also serves as an innate defense mechanism for destruction intracellular pathogens. Previous studies showed that *N. bombycis* infection inhibits host cell apoptosis, however the mechanism has not been elucidated. There are a big family of serine protease inhibitors (serpins) in the *N. bombycis*, most of which own signal peptides predicted as secreted proteins. We hypothesize that *N. bombycis* can secret serpin to inhibit host proteases in the apoptosis pathway, thus inhibiting Caspase activity and consequent apoptosis. Firstly, we found that *N. bombycis* serpin 14 (Nbserpin14) was secreted and localized in nucleus of the infected cells. Then we proved that Nbserpin14 can inhibit cell apoptosis via inhibiting Caspase 3 activity by Nbserpin14 overexpression in silkworm embryonic cells (BmE) and RNA interference, which extend the cell lifetime and facilitate the proliferation of *N. bombycis* within cell. At last, we found that the host caspase protease BmICE is the direct target of Nbserpin14 inhibition. In summary, these results elucidated the indispensable role of Nbserpin14 in inhibiting host cell apoptosis and shed new light on the pathogenesis of microsporidia.

Silver nanoparticles are effective in controlling microsporidia

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Many approaches and technologies have been developed as treatments for microsporidian, infections but effective, broad-spectrum and sustainable therapeutic approaches have not been found. Silver nanoparticles (AgNPs) have antimicrobial activity and are widely used against many different pathogens. AgNPs provide an opportunity to develop formulations that will control microsporidia. In this study, we synthesized AgNPs via a chemical reduction method and evaluated their formation, morphology and stability using transmission electron microscopy (TEM) and ultraviolet spectroscopy analysis. AgNPs damaged to the spore cell membrane and disrupted spore germination of microsporidia *Nosema bombycis*. This resulted in the release of microsporidia nucleic acids, proteins and respiratory chain enzymes. The anti-microsporidia activity of AgNPs was studied by measuring the silkworm larvae survival rate and spore genome replication after microsporidia infection. AgNPs have anti-microsporidian activity and could be effective components of formulations for treating or preventing microsporidia infection.

MICROSPORIDIA BIODIVERSITY AND PHYSIOLOGY
VIDEOS

Differences in structure and hibernation mechanism highlight diversification of the microsporidian ribosome.

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Microsporidia are unicellular fungal parasites that infect a wide range of organisms including silkworms[1], fish[2], honeybees[3], and humans. During the adaptation to their parasitic lifestyle, microsporidia have undergone drastic genome reduction. This resulted in the smallest known eukaryotic genome[5,6,7], and affected various conserved macromolecules like ribosomes[8]. Ribosome biogenesis and protein translation are energy-intensive processes[9,10]. Therefore, organisms operating under nutrient limitations are highly dependent on energy conservation via ribosomal hibernation and recycling[11]. The mechanisms of hibernation in microsporidia, however, remain poorly understood.

Here I present the cryo–electron microscopy structure of the *Paranosema locustae* ribosome, bound by the recently discovered and conserved eukaryotic hibernation and recycling factor Lso2[12]. Lso2 binds to the mRNA channel in a V-shaped conformation, blocking multiple functional regions simultaneously, and providing a reversible ribosome inactivation mechanism.

By comparing the obtained structure to the yeast and *Vairimorpha necatrix* ribosomes we furthermore showed that the P. locustae ribosome retains several rRNA elements absent in other microsporidia. Those rRNA elements, called expansion segments, are defining features of eukaryotic ribosomes[13,14,15,16]. Surprisingly, microsporidian ribosomes display a dramatic reduction in expansion segment content[17]. In one case, an expansion segment that is absent in *V. necatrix*, has been reduced in *P. locustae* to its most minimal version, a single nucleotide, which acts as an architectural co-factor stabilizing a protein-protein interface.

This study highlights the reductive evolution in these emerging pathogens and sheds light on a conserved mechanism for eukaryotic ribosome hibernation.

Four microsporidian hyperparasites of the bristle worm Pygospio elegans

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Metchnikovellids are hyperparasitic microsporidia of gregarines inhabiting the intestines of marine invertebrates, mostly polychaetes. To date there are about 30 described species, the diversity of the group remains poorly known despite the long history of the study. Our presentation is devoted to the metchnikovellid parasite fauna of the spionid bristle worm *Pygospio elegans*. Worms were collected at the silt-sand littoral zone of the White and Barents Seas, North-West Russia. Among the studied worms, the individuals infected with two gregarine species, eugregarines *Polyrhabdina* sp. and archigregarines *Selenidium pygospionis*, were rather common. Each gregarine species regularly harbors different metchnikovellids: *Polyrhabdina* sp. is the host for *Metchnikovella* incurvata and *M. spiralis*, *S. pygospionis* - for *M. dogieli* and a recently discovered but not yet described metchnikovellid. We usually observed hyperparasitic infection of only one of two gregarine species coexisting in the same worm. Sometimes we detected the mixed infections, when both species of gregarine were infected with one of two metchnikovellid species described for each of them. In rare cases, we have noticed the mixed infection of a gregarine species with both species of metchnikovellids in one worm, while we have never observed the co-infection of a gregarine cell with two metchnikovellid species. This unique parasitic system is very promising for studying the interactions between metchnikovellids and gregarines within one worm host. Supported by RSF № 19-74-20136.

Genetic diversity of microsporidia from lepidopteran insects in Russia and neighboring countries

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Microsporidia are obligate intracellular parasites of animals, most widespread in arthropods, in particular in insects. Twenty-four microsporidia-positive samples (as inferred from light microscopic analysis) of Lepidoptera sampled at different locations in Russia, Poland and Uzbekistan between 1961 and 2020 were subjected to sequencing of a portion of small subunit rRNA gene.

A large number of different species of microsporidia have been found in noctuid moths. Most of them belonged to the genera *Nosema*, *Vairimorpha* and *Endoreticulatus*. Moreover, among microsporidia from the common cutworm *Agrotis segetum* there were two isolates closely related to a microsporidium from *Eilema complanata* of the Bulgarian fauna (# KY615713) and an isolate from Poland from a distinct lineage within Nosematida, with about 91 % sequence identity to a clade of unidentified microsporidia from gammarids (Amphipoda).

In phyllopaghous pests belonging to Erebidae, several novel isolates of *Nosema* and *Vairimorpha* were also found. An isolate from *Thaumetopoea processionea* possessed a unique haplotype allocated outside Nosematidae.

In Pieridae sampled in North-Western Russia in 2020, two distinct microsporidia were found, one corresponding to *Vairimorpha mesnili* (*Nosema pieriae*) and another possibly representing *Nosema polyvora*. The latter species, characterized with ovo-cylindrical spores massively infecting Malpighian tubules and intestines of *Pieris brassicae*, was reported previously on the territory of Russia in the 70-ies of the last century.

It is expected that further analysis of the collection will reveal new forms and expand the understanding of the genetic diversity, distribution and phylogenetic relationships of microsporidia in insects.

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Novel findings of Microsporidia in predatory mites

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Predatory mites are a large group of entomophagous arthropods. Certain species are produced for pest biocontrol. The presence of microsporidia in the colonies of mites can negatively affect their development. Three species of mites of the genus *Amblyseius* (*A. sisimegis*, *A. swirski*, *A. montdorensis*) were assayed using PCR with microsporidia group-specific primers. A positive PCR signal was obtained for all three species. Sequencing of a fragment of the SSU rRNA gene showed 100% identity with *Vairimorpha occidentalis* in *A. cucumeris*. As for *A. swirskii* and *A. montdorensis* the SSU rRNA sequence was unique, showing 95% identity with microsporidia of the genera *Percutemincola* and *Pancytophaga* from soil nematodes, as well as an unidentified microsporidium, found through environmental DNA sequencing. The latter parasite might also have infected the soil nematodes in the products of biodegradation of organic waste. Microscopic examination of *A. montdorensis* samples revealed infection with microsporidia at the level of 10-20%. Spores of this type of parasite are on average about 1.5 microns and fill the bulk of the host's internal organs' tissues. The samples were fixed with 2.5% glutaraldehyde for transmission electron microscopy, which, in combination with molecular phylogenetic analysis, will serve as the basis for the taxonomic description of the new species. The research is supported by RSCF grant # 20-66-47010.

Susceptibility of beet webworm larvae to microsporidia from Lepidoptera

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The beet webworm Loxostege sticticalis (Ls) is one of the most notorious insect pests in European Russia and China. Pest outbreaks may cause serious damage to different agricultural crops such as corn, sunflowers, sugar beets, etc. According to research data over the period of 2003 to 2019, Ls can be infected by 6 species of microsporidia including pathogens atypical for Lepidoptera.

Three isolates of microsporidia recovered both from target and nontarget lepidopteran hosts within the last two years were tested: *Nosema* sp. PRSi-2019 from *Pieris rapae* (2019, China), *Nosema* sp. HAKr-2019 from *Helicoverpa armigera* (2019, Krasnodar Territory), *Nosema cf polyvora* from *Pieris brassicae* (2020, Pskov Region) and *Microsporidium* sp. LSNo-2020 from *Loxostege sticticalis* (2020, Novosibirsk Region).

Ls larvae were fed with microsporidian spores (at the dosages ranging from 3.5×105 to 8×105 spores/larva depending upon the microsporidia source and larval size) and reared in the laboratory on an artificial diet. After thirty-four days, the smears were prepared from larval tissues and examined by using bright field light microscopy (LM) or PCR.

Two isolates of microsporidia which induced infections were *Nosema* cf *polyvora* and *Microsporidium* sp. LSNo-2020. For the former parasite, 90% infection level was detected using PCR (N=20). For the latter parasite, LM showed presence of microsporidian spores in 100 % of samples (N=5), with productivity of 7-8 mln spores/larva. Microsporidia spores weren't found in larvae infected with *Nosema* sp. PRSi-2019 (N=10) as well as *Nosema* sp. HAKr-2019 (N=4).

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MICROSPORIDIA BIODIVERSITY AND PHYSIOLOGY
POSTERS

Occurence of microsporidia in trematodes infecting snails in St.Petersburg (Russia) water basins

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Hyperparasitism of microsporidia (M) is a widespread phenomenon. However, M infections in trematodes are poorly studied and very little is known about distribution and host specificity of M infecting sporocysts and rediae, developing in the host-mollusk.

The survey for trematode fauna in six species freshwater gastropods of genera *Lymnaea*, *Viviparus*, *Bithynia* and *Planorbarius* have been undertaken in 20 lakes in vicinity of St. Petersburg (Russia) in 2018–2020. Simultaneously, we examined these trematodes for hyperparasites, particularly for M. Overall, 1,566 snails were dissected. The prevalence of their infection with trematodes varied from 4% to 72% depending on locality. Twenty-eight species belonging to 9 families were identified. Microsporidian spores and developmental stages were found in seven species of trematodes. The first species, "*Microsporidium* ex. *Echinostoma* sp." was isolated from rediae of trematodes of the family Echinostomatidae. Infected echinostomatids included *Echinostoma spiniferum, E. revolutum, Echinoparyphium recurvatum,* and *Neoacanthoparyphium echinatoides*. Surprisingly, *Echinoparyphium aconiatum* belonging to the same family, remained uninfected in all studied water bodies. Moreover, the attempts to infect *E. aconiatum* rediae by feeding microsporidian spores to molluscs failed to produce the infection. Among mollusks baring echinostomatid rediae, infection with microsporidia ranged from 12% to 100%. The second M species, *Microsporidium* ex. *Diplostomum* sp., was found in the body wall cells of sporocysts of *Trichobilharzia* sp. (Schistosomatidae), *Tylodelphys clavata* and *Diplostomum pseudospathaceum* (Diplostomatidae).

Our survey for microsporidiosis among flukes inhabiting freshwater snails demonstrated the following. (1) Trematodes of the families Diplostomatidae, Schistostomatidae and especially Echinostomatidae are extensively infected with M. (2) No specificity of M in relation of the super host (gastropod) was observed. (3) Diversity of microsporidia infecting trematodes, there life cycles and pathogenicity, as well as their ability to control natural population of trematodes, demand further studies.

NEMATODE DIVISION

NEMATODES AS MODEL IN APPLIED BIOLOGY AND SOIL ECOLOGY
VIDEOS

Effect of *Bacillus thuringiensis* spores on the second stage juveniles of soybean cyst nematode.

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Soybean cyst nematode (SCN) is a plant parasitic nematode that infests the roots of soybean. Effective biological control of SCN has not yet been found, and the search for such a control method is needed. *Bacillus thuringiensis* (BT) is a spore-forming bacterium that produces crystal proteins and metabolites during spore formation. Previous studies have shown that green manure crops treated with BT spores under the plant reduce the density of SCN in the soil. In this study, we hypothesized that BT would affect the survival or mobility of SCN second stage juveniles and aimed to test this hypothesis. Two strains of BT, commercialized Jackpot® granule hydrate and *Japonesis* HSO117, were used in the experiments. The endospore suspensions of BT were exposed to the second larvae of SCN for 2 and 4 days and the survival rate was tested. Next the endospore suspension was filtered to remove the spores and endospore filtrate was prepared for the same test. Second stage juveniles exposed to the BT endospore suspension for 2 or 4 days showed significantly higher mortality in the 5% range compared to the control. In contrast, exposure to BT spore filtrate resulted in a significant reduction in the mobility of SCN second stage juveniles. These results indicated that BT could have a synergistic effect on the survival of second stage juveniles through the interaction of endospores, secondary metabolites and crystalline proteins. Further the secondary metabolites and crystalline proteins have the effect of reducing the mobility of SCN second stage juveniles.

Synergistic nematicidal activity of secondary metabolites produced by the entomopathogenic bacterium *Photorhabdus l. sonorensis* (Enterobacteriacea) against the root knot nematode, *Meloidogyne incognita* (Nematoda: Tylenchida)

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The synergistic nematicidal effects of two Photorhabdus l. sonorensis-derived secondary metabolites (SMs; AK1+AK2) against the root knot nematode, *Meloidogyne incognita* (RKN), were assessed in in planta assays. RKN-susceptible cowpea seedlings, treated with the AK1+AK2 mixture, showed a reduction in the numbers of galls and egg masses at six weeks post-treatment. Root infection was also significantly suppressed with increasing SM concentrations; however, the number of egg masses/gall was not affected. We also assessed the potential effect of these SMs on plant toxicity. Plant physiological parameters, such as leaf chlorosis and chlorophyll contents (chlorophyll *a*, chlorophyll *b*, the ratio of chlorophyll *a* and *b*, and total chlorophyll), were measured. Our results showed that only chlorophyll b and total chlorophyll were significantly reduced by the presence of RKN alone (regardless of SM concentrations). *Photorhabdus* SMs had no effect on leaf chlorosis and chlorophyll. Importantly, plant growth parameters measured (total plant dry weight, leaf thickness [leaf mass/leaf area], root thinness [root length/root mass], and root to shoot mass ratio) did not show significant responses to levels individual factors (SM concentrations or presence/absence of RKN), or their interactions (SM x RKN presence/absence). Therefore, the AK1+AK2 mixture had no phytotoxic effects on the cowpea seedlings. Our results suggest that the combined use of the AK1+AK2 synergistic mixture can antagonize RKNs in vivo by reducing the ability of this economically important plant parasite to infect the host plant roots, without impairing plant growth or chlorophyll contents through a single root application.

Characterization of Entomopathogenic Nematodes at Rapid Desiccation

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The success of a biological agent is codependent on three factors: survival, infectivity to target pest and the environmental conditions. On foliage, Entomopathogenic nematodes (EPN) undergoes rapid desiccation by reduced humidity that is detrimental to their activity and survival. However, the mechanism of rapid desiccation, its effect on survival trends and infectivity are poorly understood. The objective of this study is to characterize the varied response of EPN to rapid desiccation by survival and rate of water loss in EPN species under reduced humidity conditions. We identified that, *S. carpocapsae* was able to better adapt, and survive under rapid desiccation in comparison to *S. feltiae* and H. bacteriophora. We further evaluated the survival of *S. carpocapsae* under severe reduced humidity conditions and identified differences in the survival ability to tolerate mediocre to low relative humidity. We also compared the rate of water loss of EPN through gravimetric method and observed differences in their water loss dynamics. In addition, Attenuated total reflectance- Fourier transform infrared spectroscopy was employed to identify hydration dependent changes in the spectra of rapidly desiccated EPN. The above mentioned parameters could assist in defining the requirements of formulation to maintain optimal micro-climate on foliar surface. In addition, we propose to use this developed method as a scientific toolkit to compare and evaluate formulations for EPN foliar applications.

NEMATODES AS MODEL IN APPLIED BIOLOGY AND SOIL ECOLOGY
POSTERS

Impact of differentiated vineyard management on the activity of entomopathogenic nematodes in La Rioja (Spain)

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Current viticulture is still widely based on intensive tillage and agrochemical applications to control pests, diseases, and weeds. These practices compromise the presence of beneficial soil organisms such as the entomopathogenic nematodes (EPNs). We hypothesized that organic pest-disease management and the implementation of alternative strategies to tillage (i.e., cover crops or mulches) might enhance the EPN presence and activity. In autumn 2019, we collected two composite soil samples from 80 vineyards distributed all across the Designation of Origin (DOCa) Rioja area, selected to belong to one category of two factors: soil management (intensive tillage and alternative strategies, 48 and 32 plots, respectively) and pest-disease management (integrated -IPDM- and organic -OPDM-, 40 plots each). Using the traditional insect-bait method, we assessed three soil activity rates associated with the percentage of *Galleria mellonella* larvae that (i) die (total activity), (ii) shown nematode emergencies (nematode activity), and (iii) confirmed Koch's postulates (EPN activity). Nematode species were identified by qPCR using species-specific primers and probes. Soil management did not significantly affect any of the soil activities evaluated. Conversely, we recorded significant ($P < 0.05$) higher soil activity rates for OPDM than IPDM (11.3% vs. 4.2% for total activity, 3.2% vs. 0.4% for nematode activity, and 2.1% vs. 0.3% for EPN activity, respectively). The species Steinernema feltiae and *Heterorhabditis bacteriophora* were the most frequently identified EPN species. In conclusion, the higher EPN activities supported by OPDM may translate into increased resilience against potential arthropod pests in vineyards.

The effects of female pheromone exposure on lethal fighting in *Steinernema carpocapsae* males.

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Males of certain species of the entomopathogenic nematode *Steinernema* spp. engage in intraspecific lethal fighting. Males wrap around and compress their opponent, resulting in paralysis or death of the target. Previously, it was found that this behaviour was increased in the presence of a virgin (but not a mated) female of the same species, supporting the hypothesis that males fight for access to females. It was also previously found that males that had mated were more successful in terms of survival and killing than naïve males when the two were paired in controlled fights, but the reason for that advantage was unclear. Interpreting the advantage of mated over naïve males is complicated, since female pheromone alone results in physiological changes including sperm development in *Steinernema* males. To investigate the reason for mated male advantage in *Steinernema carpocapsae*, we paired mated males, or males exposed to female pheromone only, in staged fights against naïve males. Incidence of fighting, number of fights, average duration and latency, as well survival at 24hrs and 48hrs were recorded. The results of these experiments will be discussed in the context of the widespread effects of pheromones on nematode development and behaviour.

Unraveling the effect of the presence of earthworms or their cutaneous excreta and entomopathogenic nematodes in the soil bacterial community, biocontrol capacity, and plant traits

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Earthworms and entomopathogenic nematodes (EPNs) are well-known beneficial soil organisms that can be applied to crop soils as biofertilizers and biocontrol agents of arthropod pests, respectively. But the inoculation of new organisms may alter the soil biota composition and the functions displayed for them. To unravel how this augmentation can alter the bacterial soil community (BSC), biocontrol capacity, and plant traits over time, we investigate single and combined applications of the earthworm *Eisenia fetida* (W), or its cutaneous excreta (CEx), and the EPN species *Steinernema feltiae*, in soil mesocosms with one tomato (*Solanum lycopersicum*) plant. The treatments (n = 4) were: (i) control, (ii) W (three individuals/pot), (iii) CEx (produced by three earthworms/pot two times), (iv) EPN (3000 nematodes/pot), and their combinations (v) W+EPN, and (vi) CEx+EPN. Three exposure times were established: pre-application (T0) and post-application after two and four weeks (T1 and T2, respectively). The whole experiment was conducted twice. Overall, plant traits were not affected by the treatments. At T2, we reported higher *Galleria mellonella* larval mortality for the W+EPN and CEx+EPN treatments than for EPN single applications. Next-Generation Sequencing approaches showed a shift in the BSC from T0 (soil) to T1-T2 (soil+plants). Also, bacterial species richness was lower for the W treatment at T1. Contrary, at T2, all the treatments except W significantly increased their richness. We concluded that the augmentation with W and EPN alter soil biota and activity within a month.

Target molecules of *Bacillus thuringiensis* crystal proteins in *C. elegans*

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Bacillus thuringiensis (Bt) produces a variety of crystal (Cry) proteins during sporulation. Cry proteins have been used as agricultural pesticides against insects for decades. Our lab has pioneered work on Cry proteins, e.g., Cry5B, active against nematodes, and these are being developed for use against animal and plant parasitic nematodes. Here we are studying the mode of action and resistance of three-domain Cry proteins other than Cry5B that are toxic to nematodes.

We discovered a *Caenorhabitis elegans* mutant (bre-6) identified by conditional selection during forward genetic screens. Bre-6 was shown to show resistance to a wide range of nematicidal three-domain Cry proteins. Whole genomic sequencing was used to identify two gene candidates, a nuclear hormone receptor (gene1) and another nearby protein (gene2) in the same pathway as gene1. Extrachromosomal DNA, RNAi knockdown and the Crispr gene editing experiments confirmed that both genes are required for the resistance of bre-6 to the Cry proteins. RNAseq experiments were conducted to study downstream targets of the nuclear hormone receptor, confirming this pathway is knocked down in the mutant. Here, we will present these results as well and initial data on a new resistant mutant, bre-7, that is also apparently widely resistant to nematode-active Cry proteins. Taken together, our data suggest complex evolutionary relationships between Bt Cry proteins and nematode targets.

Transcriptomic analysis of two entomopathogenic *Steinernema* nematodes highlights metabolic costs associated with *Xenorhabdus* endosymbiont carriage.

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Entomopathogenic *Steinernema* nematodes are symbiotically associated with *Xenorhabdus* bacteria and together an antagonist partnership against their insect hosts. The association *Steinernema-Xenorhabdus* is mutualistic. The nematodes protect the bacteria from the external environment and vector them while the bacteria produce antimicrobial compounds in the insect cadaver facilitating nematode invasion and reproduction in the insect cadaver. Despite these benefits, it is yet unclear what the potential metabolic costs for Steinernema IJs associated with maintenance and vectoring of *Xenorhabdus*. We performed an RNA-seq analysis of infective juveniles(IJs) of two *Steinernema-Xenorhabdus* associations: *S. carpocapsae-X. nematophila* and *S. puntauvense-X. bovienii*. Three conditions were study: 1) IJs reared in the insect (*in vivo colonized*), 2) colonized IJs reared on liver-kidney agar (in vitro colonized) and 3) IJs depleted by the bacteria reared on liver-kidney agar (*in vitro aposymbiotic*). Our results showed differential expression between in vivo and aposymbiotic condition of the 3,457 transcripts for *S. carpocapsae* and 3,031 transcripts for S. *puntauvense*. Among these, 1,033 orthogroups of transcripts are shared between the two species. The transcripts identified as down-regulated appears involved in metabolism such as amino acid, Carbohydrate and lipid metabolism. The starch and sucrose metabolism are downregulated for both species in absence of symbionts. Glycogen have an important role in the energy storage in the IJ stage and thus longevity and infectivity of the nematode. To summarized, in absence of insect host and bacteria symbiont, our transcriptomic analysis suggest that number metabolism pathways, in particular involved in the energy, are down-regulated suggesting their important role.

ADVANCES IN FORMULATION, APPLICATION AND CONTROL OF PESTS
VIDEOS

Optimisation of the in vitro liquid culture process of Steinernema yirgalamense and Steinernema jeffreyense using local resources for cost-effective production

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The in vitro liquid mass production of entomopathogenic nematodes is an expensive process. The cost of production can be substantially decreased by sourcing cheaper and locally sourced ingredients of the in vitro liquid medium diet. Results here show that high yields can be maintained when using cheaper ingredients, and can even significantly increase yields. Two local South African EPN species were used and the dietary medium replaced with cheaper and locally sourced ingredients

For both species, two alternative protein sources, egg yolk and milled insect protein were used in place of the usual soy powder protein source, to assess the impact of protein sources in the final infective juvenile yield. A second trial was also conducted for the lipid source of the medium diet, using much cheaper canola oil versus the usually expensive olive oil. Finally, a trial on the yeast component of the diet comparing expensive laboratory-grade yeast extract with low-grade brewers' yeast and Marmite. A full cost analysis for each alternative ingredient was conducted and showed how high yields can be obtained with far cheaper dietary ingredients and highlights the need for optimisation of the in vitro liquid mass culture process of EPNs

Potential of entomopathogenic nematodes to mitigate the insect vector of the Syndrome de Basse Richesse in sugar beet

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In Europe, sugar beet is under enormous pressure; threaten by several pathogens and viruses dramatically affecting the crop yield. First described in France, the Syndrome de Basse Richesse is an emerging sugar beet disease. It is induced by a Gram + bacterium (*Arsenophonus phytopathogenicus*). The bacteria are transmitted by the planthopper *Pentastiridius leporinus*. As a piercing-sucking insect, the vector releases the bacteria in the phloem, from where it colonizes the vascular system and induced necrosis. The most obvious symptoms are the yellow leaves of infected plants. Reflecting from lower photosynthetic efficacy, this is yet not the most adverse impact on the beet. Indeed, what makes the Syndrome de Basse Richesse so impactful on sugar beet yield is the dramatic reduction of sugar content in the tap root. As often, it seems easier to manage the vector than the pathogen itself and we therefore explored the potential of entomopathogenic nematodes to control *P. leporinus*. Preliminary results show infectiousness differences between species. *Steinernema feltia* was able to infect only ca. 40% of *P. leporinus* nymphs whereas *Heterorhabidits bacteriophora* killed 100% of the insect juveniles. In addition, in a 6-arm olfactometer experiment, *H. bacteriophora* was attracted toward sugar beet plant fed on by nymphs. This nematode species might therefore be a good candidate in the growers' toolbox to manage *P. leporinus* and hence the Syndrome de Basse Richesse. Of course, more data is needed to support strongly this postulate but entomopathogenic nematodes could soon be an effective tool sustainably supporting the sugar industry.

Entomopathogenic nematodes applied as infected *Galleria mellonella* cadavers against wireworms (Coleoptera: Elateridae)

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Wireworms (Coleoptera: Elateridae), are important pests of cereal crops in Montana, USA. Because of lack of effective control measures, alternative control methods such as biological control with entomopathogenic nematodes (EPNs) are needed. EPNs are normally applied in aqueous suspensions but can also be applied as EPN-infected host cadavers. Imidacloprid, a prophylactic seed treatment insecticide causes short term suppression of wireworms. This study was focused on evalauting efficacy of EPNs applied via *Galleria mellonella* L. (Lepidoptera: Pyralidae) infected cadavers in conjunction with imidacloprid seed treatment against sugarbeet wireworm, *Limonius californicus* (Mannerheim) in field and greenhouse. *Galleria mellonella* cadavers were prepared by inoculating live larvae with 200 freshly produced EPN infective juveniles. None of the EPN strains tested were found effective in terms of reducing wireworms and protecting crop yield in field. When applied with imidacloprid treated wheat seeds in greenhouse, one *G. mellonella* cadaver infected with *Steinernema carpocapsae* (All and Cxrd strains) or S. riobrave 355 killed 50-68% of *L. californicus*. The mortality range was 40-56% for *S. carpocapsae* (All and Cxrd strains) and S. riobrave 355, when seeds were planted without imidacloprid, not differing significantly from imidacloprid treatment (but higher than control). However, plant damage was significantly lower in imidacloprid treated plants (8-24%) as compared to non-imidacloprid plants (57-75%) at 35 days after treatment (DAT). *Limonius californicus* larvae reduced 57% and 92% of non-imidacloprid plants at 14 and 35 DAT, respectively. Overall, EPN alone treatment was not found as effective as the combination of imidacloprid and EPN treatments in greenhouse.

Keywords: Wireworms, *Limonius californicus*, Entomopathogenic nematodes, Montana, imidacloprid, *Galleria*

Biocontrol with Benefits: Control of Peachtree Borer with Entomopathogenic Nematodes

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The peachtree borer, *Synanthedon exitiosa*, is a major pest of peaches and other stone fruits. Entomopathogenic nematodes, particularly *Steinernema carpocapsae*, provide high levels of biocontrol efficacy against peachtree borer. Control levels following applications of *S. carpocapsae* are equal or superior to standard chemical insecticides such as chlorpyrifos. For growers that lack irrigation, a gel formulation "Barricade" can be used to facilitate nematode survival and biocontrol efficacy. In this project, we explored 1) optimization of rates and formulation, 2) whether S. carpocapsae application targeting peachtree borer provides other benefits to the grower, specifically control of root-feeding weevils. Experiments were conducted over two years in a commercial peach orchard in Georgia, USA and on the USDA-ARS research station in Byron, GA; *S. carpocapsae* was used in all experiments. Results indicated that a lower rate of 0.5 million infective juveniles per tree provided similar results to a higher rate (1.5 million per tree). Additionally, nematodes combined with a lower rate of Barricade (2%) provided similar control levels compared with the full Barricade rate (about 5%). Applications of *S. carpocapsae* were more effective than chlorpyrifos in controlling peachtree borer in the Year 1 and provided statistically similar results to chlorpyrifos in Year 2. The nematode applications targeting peachtree borer also reduced populations of root-feeding weevils such as *Oedophrys hilleri*. Our findings indicate that lower rates of nematodes and gel formulation can be used for effective biocontrol of peachtree borer, and that additional benefits can be gained when targeting peachtree borer with *S. carpocapsae*.

ADVANCES IN FORMULATION, APPLICATION AND CONTROL OF PESTS
POSTERS

Screening of adjuvants to enhance the entomopathogenic nematode survival and adherence after aerial application on grapevine leaves

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Expanding the available biological control agents to fight against aerial grapevine pests can provide new strategies in both IPM and organic viticulture. Current application technology supports the implementation of entomopathogenic nematodes (EPNs) against aerial pests. We hypostatized that by selecting the best combination of EPN-adjuvant, we can enhance EPN viability and persistence on grapevine leaves, and herewith apply EPN against aerial grapevine pests. The aim of this study was to screen for the best adjuvant-EPN mix to ensure high survival, viability, and adherence on leaves. The compatibility of five commercial adjuvants approved for grapevine application (Multi-US, MaxiMix, Dash HC, Nu-film-17 and Adrex) and four EPN populations (*Heterorhabditis bacteriophora* VM-21, *Steinernema carpocapsae* ALL, *S. feltiae* RM-107 and *S. feltiae Koppert*) were tested by exploring the maximum adjuvant recommended field dosage concentration. In laboratory experiments, we investigated the survival of infective juveniles (IJs) after 4 and 24 h exposure to each adjuvant. Thereafter, we tested their infectivity against the model insect *Galleria mellonella* (Lepidoptera: Pyralidae). Adherence to leaves and survival was determined by spraying the IJs-adjuvant combination on grapevine leaves and establishing the leave coverage and their survival after 1 h. Overall, all the EPN populations were compatible with all the adjuvants, except for *H. bacteriophora*-Adrex combination. Also, most of the adjuvants provided similar to control leave coverages, maintaining IJs survival ~80% after 1 h. Additional studies are needed to increase the grapevine leave coverage by EPN. These promising results bring new opportunities to apply EPN against aerial grapevine pests.

Potential of entomopathogenic nematode isolates from Germany and Israel to control the tomato leaf miner(Tuta absoluta, Meyrick)(Lepidoptera:Gelechiidae) in Georgia

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The most important, damage cause of pests in Georgia today is Tomato leaf miner(*Tuta absoluta, Meyrick*),(*Lepidoptera:Gelechiidae*). Tuta absoluta lives of tomato on and in the leaves, and also in the fruit of tomatoes, and as it can cause up to 100% yield loss.

Species of the entomopathogenic nematodes, Steinernema carpocapsae isolate "Zi" (Germany) and strain *Steinernema carpocapsae* (Israel) were studied against Tomato leaf miner, Tuta absoluta (Meyrick). Under laboratory conditions, by using different nematode strains 500 juveniles IJ/ml of *S. carpocapsae* isolate "Zi"(Germany) and strain *S. carpocapsae* (Israel) were used separately and in combination against Tomato leaf miner, 4th instar larvae and pupae Tuta absoluta. In both strains were used water for control. In the results of investigation to shown, that high effect of all treatments of pests such as, 4th instar larvae of Tomato leaf miner,*Tuta absoluta* was recorded on the 7th day, after treatments with *S.carpocapsae* izolate Zi(Germany) and *S.carpocapsae* (Israel) in combination. Separately the percentages of 4th instar larvae mortality were 65 % for S.carpocapsae izolate Zi, 78 % for *S.carpocapsae* (Israel). In combination of both strains *S.carpocapsae* izolate Zi,(Germany) and *S.carpocapsae* (Israel) were 94%.

Separately the percentages for pupae mortality for *S.carpocapsae* izolate Zi,(Germany) was 47 %, for *S.carpocapsae* (Israel). 62%, in combination of both strains were 71% and no larvae mortality on control treatments.

In conclusion, It was determined that, 4th instar larvae *T.absoluta*(Tomato leaf miner) can be controlled by *S.carpocapsae* izolate Zi (Germany) and *S.carpocapsae* (Israel) in the combination and further studies should be conducted at field and greenhouse conditions.

Performance of Steinernema glaseri pre-conditioned IJs formulated as pellets with sodium poliacrilate

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Entomopathogenic nematode (EPN) formulations in pellets show a shorter survival time than other formulations such as alginate capsules or cadavers of *Galleria mellonella*. However, the pellet has the advantage of having a microstructure, which can be reproduced consistently to maintain quality through a mechanized process. With the formulation in diatomaceous earth pellets the viability of IJs of *S. glaseri* has been extended to 2 months, but it has been observed that the pathogenicity on *P. vetula* decreases significantly. The design of the pellet to promote the drying and oxygenation regimes required to induce anhidrobiosis in IJs, as well as pre-conditioning, can be strategies to improve the survival and infectivity of the NEP. To evaluate the survival and infectivity of two species of nematodes formulated in pellets of different particle size and porosity, laboratory experiments were carried out with larvae of *G. mellonella*. *Tenebrio molitor* larvae were subsequently used to evaluate the same parameters in preconditioned IJ of *S. glaseri* formulated in pellets with sodium polyacrylate. The treatment with Soil (S)-Ash (C)-Diatomaceous earth (D) at percentages of S15-A50- C15-D20 kept the pelletized IJs alive for 30-40 d. It was found that the replacement of the small Petri box of the White Trap with a Petri dish containing a layer of plaster of Paris allows the Pre-IJ to finish its development in 2-3 days after emerging, which improved survival by 33.3 %. The incorporation of sodium polyacrylate into the pellet increased its water retention capacity, therefore it may improve moisture availability.

Steinernema carpocapsae and *Xenorhabdus nematophila* based products for the control of the grapevine moth and the grey mold in vineyards

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The European grapevine moth (EGVM) *Lobesia botrana* (Lepidoptera: Tortricidae) is a relevant pest in the Palearctic region vineyards but also present in America. Besides direct berries predation, its larval instar favors bunch rot development. EGVM is a *Botrytis cinerea* vector, responsible for the grey mold disease. Their biological control would reduce intensive pesticide use and could limit the invasion by new threats. Thanks to recent advances in applied technologies, using entomopathogenic nematodes (EPNs) for EGVM control is nowadays a real option. Thus, cell-free supernatants (CFS) derived from EPN-symbiotic bacteria are arising as powerful bio-tools against aerial insect pests and certain diseases. This study aimed to determine the viability of *Steinernema carpocapsae* and *Xenorhabdus nematophilus* CFS applications against different larval stages of EGVM and their impact on *B. cinerea*. *In vitro* experiments showed higher larval mortality rates for L5 (100%) than in L3 (79%) and L1 (63%) after five days of inoculation with 10 infective juveniles (IJs)/cm2. For pupae, we recorded 52% of mortality when exposed to 50 IJs/cm^2 after fourteen days. The *X. nematophilus* CFS applications supposed 96% and 92% mortality rates for L1 and L3, respectively, after five days. Similarly, we observed 67% growth inhibition for *B. cinerea* in Potato Dextrose Agar (PDA) plates after three days of 10% *X. nematophilus* CFS's application. Our findings prove the potential of EPNs and CFS derivate as control methods against EGVM and *B. cinerea*. More studies involving other species will improve our knowledge of this pest/disease complex.

VIRUS DIVISION

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VIDEOS

Drosophila as a model to identify viral envelope protein trafficking pathways

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Viral envelope protein trafficking to the plasma membrane is critical for egress and successful replication of most enveloped viruses. To analyze envelope protein trafficking in insect cells, we used a baculovirus (AcMNPV) envelope protein (GP64) for studies in both cultured cells and the midgut epithelium of *Drosophila melanogaster*. To identify the requisite cellular factors for general trafficking to the plasma membrane, we developed a stable Drosophila cell line that inducibly expresses the GP64 envelope protein and performed a targeted RNAi screen of 250 host genes known to be involved in vesicular trafficking. We identified 12 cellular genes (encoding Rab GTPases, Rab effectors, clathrin adaptors and SNAREs) that were important for GP64 transport and display on the cell surface. We also examined the effects of knockdowns of these key genes on intracellular localization of GP64. To investigate viral envelope protein trafficking in polarized midgut cells, we developed transgenic Drosophila fly lines that inducibly express baculovirus GP64 in the midgut, and found that expression of GP64 in the absence of other viral proteins, was sufficient for polarized trafficking to the basal membranes of midgut cells where virion budding occurs. We are similarly assessing polarized trafficking of the vesicular stomatitis virus G protein in transgenic Drosophila as a model for arbovirus envelope protein trafficking in midgut and salivary gland cells.

Identification and Tissue tropism of newly identified iflavirus and negevirus in tsetse flies *Glossina morsitans morsitans*

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Tsetse flies cause major health and economic problems as they transmit trypanosomes causing sleeping sickness in humans (Human African Trypanosomosis, HAT) and nagana in animals (African Animal Trypanosomosis, AAT). One solution to control the spread of these flies and HAT is the Sterile Insect Technique (SIT), whose success relies on establishment and maintenance of healthy and competitive insect colonies. However, mass production of tsetse is threatened by covert virus infections, such as the *Glossina pallidipes* salivary gland hypertrophy virus (GpSGHV), a large, rod-shaped dsDNA virus. This virus infection can switch from a covert asymptomatic to an overt symptomatic state, which can cause the collapse of an entire fly-colony. Meanwhile, GpSHGV can be managed reasonably well, but the identification of additional covert viruses poses other viral threats to tsetse colonies. Recently, we demonstrated the presence of an iflavirus and a negevirus, both positive sense, single stranded RNA viruses, in *Glossina morsitans morsitans*. Besides *G. m. morsitans*, other *Glossina* species in the Seibersdorf rearing facility (Insect Pest Control Laboratory, IAEA, Austria) have been shown to carry both viruses. In the current study we assessed the prevalence of the iflavirus and negevirus in different teste fly species. We analyzed the tissue tropism of these viruses in *G. m. morsitans* hosts to decipher their mode of transmission. Our results demonstrate the presence of both viruses in host tissues such as the brain and fat bodies, but also in their reproductive organs (ovaries and testes), milk and salivary glands, suggesting potential viral transmission either horizontally during feeding and/or vertically from parents to offspring. This study provides the first biological data on the potential effects of iflavirus and negevirus on tsetse colonies.

Bombyx mori Pupae Efficiently Produce Recombinant AAV2/HBoV1 Vectors with a *Bombyx mori* Nuclear Polyhedrosis Virus Expression System

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Recombinant adeno-associated viral vectors are currently the most popular gene therapy vectors due to their non-pathogenicity, low immunogenicity, and long-term stable expression of exogenous genes. The baculovirus-insect cell/larvae expression system has shown great potential in virus production. In order to establish a more efficient production system, *Bombyx mori* larvae and pupae were used as a new platform and infected with recombinant *Bombyx mori* nuclear polyhedrosis virus (BmNPV). Two recombinant rBmNPV (rBmNPV/AAV2Rep-HBoV1Cap and rBmNPV/AAV2ITR-eGFP) were constructed for efficiently produce AAV2/HBoV1 chimeric vector by co-infection. The yield of rAAV2/HBoV1 derived from rBmNPV/AAV2Rep-HBoV1Cap and rBmNPV/AAV2ITR-eGFP co-infected BmN cells exceeded 2×10^4 vector genomes (VG) per cell. The rBmNPV was shown to be stable and showed no apparent decrease in protein expression after five passages. Significantly, rAAV2/HBoV1 could be efficiently generated from rBmNPV-infected silkworm larvae and pupae with an average yield of 2.52×10^{12} VG/larva and 4.6×10^{12} VG/pupa, respectively. However, the high rate of empty shells in the vectors generated from larvae and pupae suggests that the platform needs further optimization in the future. Our work shows that silkworm pupae, as an efficient bioreactor, have great potential for application in the production of gene therapy vectors.

Generation and characterization of the AcMNPV-Bombyx mori bidensovirus chimeras

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Bombyx mori bidensovirus (BmBDV) is currently the only species in the family of *Bidnaviridae*. Its genome contains two linear single-stranded DNA segments of 6.5 kb (VD1) and 6 kb (VD2). Since there is no in vitro cell line to support BmBDV infection, the research on the proliferation and gene function of BmBDV is greatly restricted. In this study, we fused the Flag tag at the NS1 and VP ends of VD1, and inserted eGFP gene into the non-coding region of the VD2 genome, and then the chimera AcMNPV-BmBDV viruses Ac-VD1/NS1Flag, Ac-VD1/VPFlag and Ac-VD2GFP were constructed, respectively. The recombinant viruses were infected with Hi5 cells, and the cells were lysed to feed sensitive strains of silkworm larvae. Both RT-PCR and Western Blot results indicate that the BmBDV genome can be transcribed and expressed in cells infected with the chimeric virus. However, no symptoms of infection were observed in the silkworm fed with lysate, and no related protein expression was detected, indicating that BmBDV was not rescued with the replication of recombinant virus in Hi5 cells. Southern Blot results also showed that BmBDV genome could not be rescued by chimera viruses. Our research provides a convenient platform for in-depth exploration of the expression regulation of BmBDV, and lays the foundation for the creation of new biological control agents such as chimeric viruses.

Functional and Morphological Analysis of Invertebrate Iridescent Virus 6 (IIV6) Potential Matrix Protein (415R)

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Invertebrate iridescent virus 6 (IIV6) encodes 215 putative open reading frames (ORFs). Interactions among the 54 structural proteins, investigated using yeast two-hybrid system, revealed that 415R interacts reciprocally with the potential envelope protein 118L and the major capsid protein 274L. This result suggested that 415R may be a matrix protein that plays a role as a bridge between the capsid and envelope proteins.

Recombinant IIV6 (rIIV6Δ415R-GFP) was generated by replacing a green fluorescent protein (gfp) gene instead of 415R ORF. Resulted recombinant IIV6 could not be purified from wild-type virus. This result showed us that the virus could not reproduce in the absence of 415R protein. Subsequently, 415R gene was silenced with specific dsRNA's. Results showed that the titer of the progeny virus in dsRNA transfected cells was 50% lower than those in cells without dsRNA transfections. Specific antibodies produced against 415R protein were used to determine the location of the 415R protein in the virion structure. Both western blot hybridization and immunogold electron microscopy analysis showed that the 415R protein was found with/on virion structures treated with Triton X-100 which degrades the viral envelope.

These results provide important data on the role and location of IIV6 415R protein in the virion structure. Additionally, these results may also shed light on the identification of the homologs of 415R among the vertebrate iridoviruses. Identifying matrix protein homologs of vertebrate iridoviruses will be helpful at the control of these viruses that cause deaths, especially in fish and frogs.

Construction of a vector for expression of recombinant proteins in in insect cells' mitochondria.

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Baculoviruses are insect viruses used as bioinsecticides and as one of the most versatile vectors for the expression of recombinant proteins in eukaryotic (insect) cells. Bacillus thuringiensis (Bt) comprises a large group of facultative aerobic bacteria capable of producing crystalline proteins, called δ-endotoxins (*Cyt2Ba* and *Cry11A* proteins) which are toxic to different insects' species. We have constructed a recombinant baculovirus containing eGFP reporter gene fused to an insect mitochondrial localization signal sequence (MLS) taken from a binding protein to the juvenile hormone of Bombyx mori (GeneBank accession NP_001040474). Recombinant virus containing the *eGFP* were used to infect insect cells and at different times post-infection, and their presence inside mitochondria was confirmed by confocal microscopy. We then constructed recombinant baculoviruses containing *Cry11A* and *Cyt2Ba* genes fused or not to the MLS and in the presence of a Bt chaperone gene (p20). The expression and crystal-forming proteins were analyzed by light microscopy, transmission electron microscopy (MET), and Western blotting. An amorphous crystal structure was observed inside mitochondria by light and electron microscopy. We confirm the expression of *Cry11A* and *Cyt2Ba* proteins by Western blotting using antibodies against *Cry11A* and *Cyt2Ba* proteins. When we expressed the *Cry11A* protein together with the chaperone p20, we observed a better formation of the crystals in insect cells. These recombinant proteins will be used in bioassays to confirm their toxicity towards insect larvae.

A functional peroral infectivity complex is present in the envelope of White Spot Syndrome Virus of shrimp

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White Spot Syndrome Virus (WSSV) is a major cause of disease in shrimp culture worldwide. The virus infection process is well studied, but the entry mechanism is a matter of debate. The major virion envelope protein VP28 has been implied in the oral and systemic infection of the virus. However, genetic analysis of the viral DNA has shown the presence of a few genes related to a so-called oral infectivity factor (PIF) complex as in baculoviruses. This complex is essential for virion entry in midgut epithelial cells of insect larvae. So, the question is whether a PIF complex exists in WSSV, what the components of this complex are, whether it functions as an oral infectivity complex in shrimp and what biochemical properties contribute to its function in a marine environment. The results indicate that indeed WSSV has a PIF complex (≈720 kDa) consisting of at least eight components, four of which were not identified as PIF proteins before. The complex is resistant to alkali, proteolysis and high salt, properties that are important for maintaining virion integrity and infectivity in the marine environment. Oral infection can be neutralized by some PIF-specific antibodies, but not by a VP28-specific antibody. The results indicate that the PIF complex is essential for oral infection of shrimp by WSSV. The presence of a PIF complex in WSSV, which is ancestrally related to baculoviruses, nudiviruses, hytrosaviruses, and bracoviruses of invertebrates, may suggest that this complex is functionally highly conserved in disparate invertebrate virus taxa.

ADVANCES IN INSECT MOLECULAR VIROLOGY
POSTERS

Equivalence of Cypoviruses α-Helixes : Evidence of Convergent Evolution of Structure and Function

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Cytoplasmic polyhedrosis viruses (CPVs) are insect viruses belonging to the genus *Cypovirus* within the family Reoviridae. Coded by its segmented dsRNA, a protein called polyhedrin composes a great part of the polyhedra, a proteinaceous crystal in which cypovirus virions are embedded. The molecular organization of the polyhedra consists in tetrahedral clusters of four polyhedrin trimmers, connected by non-covalent interactions mainly mediated by the N-terminal a-helix (H1). Therefore, it is possible to associate proteins of interest to the polyhedra fusing them with H1. In this work, we aimed to evaluate if the H1 of Thyrinteina arnobia CPV (TharCPV), could be used for the association of the Sars-CoV-2 RBD to the polyhedra of Chrysodeixis includens CPV (ChinCPV), a new cypovirus sequenced by our group, instead of its own H1. For this, a SDS PAGE was performed using polyhedra from each cypovirus and the results were analysed by densitometry. The H1 of TharCPV had the ability to associate the antigen to both crystals, with no significant difference in efficiency, even though the crystals differ in size and shape. In addition, in silico analysis were performed, aligning the H1s from TharCPV and ChinCPV in their primary and secondary structures. Despite the H1s only sharing 20% of their amino acids, their secondary structures are almost identical, indicating that a convergent evolution made them equivalent. A heterologous prime-boost immunization could be done with 2 types of polyhedra with the same antigen associated, an excellent type of immunization.

Separating small extracellular vesicles from baculovirus virions

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Extracellular vesicles (EVs) are key messengers between cells, transporting proteins and RNA to recipient cells, which may alter their actions. Small EVs from cells infected with HIV or herpes simplex virus, among others, were found to illicit pro- or antiviral reactions in non-infected cells. It would be interesting to study if small EVs play a similar role in insect-virus interactions, which can be studied in the baculovirus infection in *Spodoptera frugiperda* cells. Firstly, to identify the effect of small EVs from infected cells upon naïve cells, it is essential to separate virions from small EVs. To this end, size exclusion chromatography was combined with a two layer sucrose density gradient ultracentrifugation.

Size exclusion chromatography separated small EVs and virions from proteins and other small molecules. Various sucrose concentrations and ultracentrifugation times were tested. Nanoparticle tracking analysis showed two particle peaks, located at the interphase between the two sucrose concentrations and at the top of the gradient. Plaque assay and qPCR analyses showed the vast majority of virions were concentrated at the interphase. Western blot for the major viral glycoprotein GP64 showed it was abundant at both particle peaks. TEM analysis showed baculovirus mainly at the interphase and small EVs both at the interphase and the top of the gradient. Together these results indicate these methods are able to isolate small EVs from the vast majority of the baculovirus virions. Provided the size and density are not too close, these methods may be optimised to separate other virions from small EVs.

BACULOVIRUS REPLICATION AND MORPHOGENESIS
VIDEOS

Hyper-expression of baculovirus P10 and processing by viral cathepsin are required for nuclear disintegration and release of polyhedra from Autographa californica multiple nucleopolyhedrovirus-infected cells

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Baculovirus infection often leads to death and post mortem liquefaction of insect larvae. This is essential for polyhedra release and subsequent horizontal virus transmission. Liquefaction depends on virus-encoded P10, v-cathepsin and chitinase. Chitinase serves as a chaperone for v-cathepsin processing to the active form and probably serves to break down chitin in the insect cuticle. However, the role of P10 in the liquefaction process is less certain. Microscopy studies showed that P10 forms cytoskeletal-like structures in the cytoplasm and nucleus. During early P10 formation, the cytosolic filamentous structures interact with microtubules, which might act catalytically in facilitating P10 structures. Subsequently, thick rod-shaped P10 tubules form around the nucleus. This "cage"-structure remodels to a polarised P10 mass before nuclear disintegration and polyhedra release occur. This event appears to be accompanied by cleavage of P10. P10 or v-cathepsin but not chitinase-deficient AcMNPV failed to induce nuclear lysis and polyhedra release, although the cytoplasm was disrupted. Inhibition of cysteine protease activity by E64 blocked polyhedra release and P10 cleavage. Mutations of the potential cathepsin cleavage-site within P10 reduced polyhedra release but also levels of P10. Reconstitution experiments, where v-cathepsin was added to monolayers of residual nuclei of cells infected with a v-cathepsin-deficient AcMNPV resulted in P10 processing, nuclear lysis and release of polyhedra. We suggest a putative mechanism in which perinuclear P10 cleavage by v-cathepsin and P10 hyper-expression promotes remodelling of P10 structures resulting in nuclear disintegration and release of polyhedra.

Autographa californica Multiple Nucleopolyhedrovirus Ac16 modulates the accumulation of IE1

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Autographa californica multiple nucleopolyhedrovirus (AcMNPV) Ac16 (BV/ODV-E26) acts as a regulator of the level of IE0 and IE1. However, it is not clear how Ac16 regulates IE1 in infected cells. In this study, bioinformatic analysis showed that Ac16 contains an nucleolar localization signal (NoLS). Truncation experiments and mutation analysis demonstrated that the NoLS fused to GFP could localize to nucleoli, but Ac16 was co-localized in nuclei with IE1 early in AcMNPV-infected cells. An ac16-null AcMNPV bacmid was constructed, and ac16 under the control of ie1 promoter was inserted into the ac16-null AcMNPV bacmid, surprisingly the construct abrogated viral DNA replication and virus production. Amino acid sequence analysis showed that the residues 56-148 are highly conserved in all Ac16s, mutation analysis demonstrated that amino acids 60-61 or 71-74 are important for interaction between Ac16 and IE1. Two Ac16 proteins with mutations at residues 60-61 or 71-74 under the control of ie1 promoter were inserted into the ac16-null AcMNPV bacmid, the constructs containing the point mutations in AcMNPC

The Autographa californica Multiple Nucleopolyhedrovirus ac26 Gene Is Critical for Morphogenesis of Occlusion Body

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Occlusion bodies (OBs) of nucleopolyhedroviruses are well-ordered protein lattices which are important for protecting the occlusion-derived virions (ODVs) from environmental degradation factors. The mechanism of OB morphogenesis remains unclear. In this study, we found that the deletion of *ac26* in Autographa californica multiple nucleopolyhedrovirus (AcMNPV) led to striking abnormities of OBs including cubic shape, enlarged size, as well as a significant decrease in OB numbers. Repair of *ac26* rescued the abnormalities; indicating *ac26* is critical for the morphogenesis of OBs. *Ac26* was identified as a late gene, encoding a 14.6 kDa protein, which presented in the OB matrix and ODVs, but not in the budded virions (BVs). Crystallographic structural analysis of a large OB (~10 μm in diameter) generated by the ac26 knockout virus revealed the absence of the disulfide bonds and a partial interruption of the nearby salt bridges between trimmers of polyhedrin within a unit cell. Mutations of 3 conserved amino acids in *Ac26* individually showed similar effects on OB morphogenesis, suggesting they are important for the function of *ac26*. The above findings revealed the connection between *ac26* with OB morphogenesis, while the model of action needs to be further studied.

Identification of differential genes in primary infection of *Spodoptera frugiperda* (Lepidoptera:Noctuidae) with an SfNPV baculovirus

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Spodoptera frugiperda (Lepidoptera: Noctuidae) is considered the main pest of maize in Mexico and Latin America, and for its control, chemical insecticides are used mostly, but there are biological control methods such as baculoviruses. The use of a single nucleopolyhedrovirus strain called SfNPV-Ar has shown good performance in the laboratory and in the field to control the population of this pest. It is considered that there are molecular mechanisms that allow this strain of virus to efficiently control the cellular machinery of its host insect and take advantage of it for its benefit. In this work, we analyzed which genes were being regulated in the columnar cells of the midgut of *Spodoptera frugiperda*, infected with this baculovirus. For this, 151 genes at different times post-infection (6, 12 and 24 h) were sent to be sequenced, assembled with the SeqMan program (DNA STAR), and contrasted with the NCBI database, to finally perform a search for its possible functions to determine its role during viral infection in the intestines of *S. frugiperda*. It was possible to identify genes regulating apoptosis, regulators of the cytoskeleton, regulators of the cell cycle, regulators of RNA transport, and reactive oxygen species. Elucidating how these genes affect the development of the insect and favor the reproduction of the virus is vital to understand why some strains are more virulent than others and have greater potential to control the armyworm of the maize.

Identification of *Spodoptera frugiperda* importin alphas that facilitate the nuclear import of Autographa californica multiple nucleopolyhedrovirus DNA polymerase

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Proteins containing nuclear localization signals (NLSs) are actively transported into the nucleus via the classic importin-α/ß-mediated pathway, and NLSs are recognized by members of the importin-α family. Most studies of insect importin-αs have focused on Drosophila to date, little is known about the importin-α proteins in Lepidoptera insects. In this study, we identified four putative importin-α homologues, *Spodoptera frugiperda* importin-α1 (SfIMA1), SfIMA2, SfIMA4 and SfIMA7, from Sf9 cells. Immunofluorescence analysis showed that SfIMA2, SfIMA4 and SfIMA7 localized to the nucleus, while SfIMA1 distributed in cytoplasm. Additionally, SfIMA4 and SfIMA7 were also detected in the nuclear membrane of Sf9 cells. SfIMA1, SfIMA4 and SfIMA7, but not SfIMA2, were found to associate with the C terminus of AcMNPV DNA polymerase (DNApol) that harbours a typical monopartite NLS and a classic bipartite NLS. Further analysis of protein–protein interactions revealed that SfIMA1 specifically recognizes the bipartite NLS, while SfIMA4 and SfIMA7 bind to both monopartite and bipartite NLSs. Together, our results suggested that SfIMA1, SfIMA4 and SfIMA7 play important roles in the nuclear import of AcMNPV DNApol C terminus in Sf9 cells.

Both the enzymatic- and structural properties of Autographa californica multiple nucleopolyhedrovirus (AcMNPV) protein tyrosine phosphatase (PTP) are insignificant for brain entry in *Spodoptera exigua* caterpillars

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Parasitic alteration of host behaviour can be caused by a broad range of organisms. Only for a few of these parasites it is known that they manifest and alter these behavioural-changes from the central nervous system (CNS) itself, and little is known about the mechanisms behind these alterations. Neuroparasitology intertwines the existing fields of neurology, biology and parasitology – covering the cases of parasitic manipulation of the CNS. After infection by baculoviruses, infected caterpillars climb the vegetation in "tree-top"-disease, and/or express hyperactivity. Baculoviral infections result in liquefaction of the caterpillars and release of virus progeny. Both behavioural alterations are thought to increase the chance of transmission to susceptible hosts. Previous studies have shown that for a subset of baculoviruses the viral protein tyrosine phosphatase (PTP) is required to induce hyperactivity.

Here, we studied baculoviral entry into the CNS of 3rd instar *Spodoptera exigua* caterpillars using a fluorescently tagged Autographa californica multiple nucleopolyhedrovirus (AcMNPV). Using different viral constructs (with PTP, without PTP or with a catalytically inactive PTP) we show that the enzymatic function of PTP is not mandatory for CNS-entry, neither is the presence of PTP as such. Furthermore, we will elaborate on the observed patterns of localization of the different virus-constructs within the CNS.

The role of BmNPV Bm65 protein in the repair of ultraviolet-induced DNA damage

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Baculovirus is a type of double-stranded DNA virus with envelope, which specifically infects invertebrates, mainly Lepidoptera, Hymenoptera and Diptera. Ultraviolet (UV) light is one of the factors that causes baculovirus inactivation. However, little is known about the response of baculoviruses to UV light. Bombyx mori nucleopolyhedrovirus (BmNPV) orf 65 (Bm65) belongs to the GIY-YIG endonuclease superfamily. In the present study, the role of viral protein Bm65 in the repair of UV damage was analysed. The results indicated that Bm65 accumulated in the nucleus and repaired UV-damaged DNA. qPCR results also showed that the expression of Bm65 genes were significantly increased when UVC-treated BmNPV or UVC-treated BmN cells were used for the analysis of viral infection. This study indicated that Bm65 was involved in repair of UV-induced DNA damage of host and BmNPV.

Keywords: Bombyx mori; BmNPV; Bm65; UV radiation; DNA damage repair

BmNPV induces cell cycle arrest and enhances viral replication by depleting BmCDK1 and BmCyclinB

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Understanding virus-host interaction is important for delineating the mechanism involved in viral replication and host resistance. Baculovirus, an insect virus, can arrest the cell cycle in the S or G2/M phase. However, the role and mechanisms of Baculovirus-mediated regulation of cell cycle progression are undetermined. Our results, obtained using flow cytometry (FCM), tubulin-labeling, BrdU-labeling and MTS, show that *Bombyx mori* nucleopolyhedrovirus (BmNPV) arrested the cell cycle in the G2/M phase and inhibited cell division, cellular DNA replication and cell proliferation. Furthermore, we found that BmNPV induced G2/M arrest to support its replication and proliferation, which was associated with BmCDK1 and BmCyclin B. Importantly, we determined that the mechanism of BmNPV LEF-11 and IAP by interacted with BmCyclinB and BmCDK1, arrest cell cycle in G2/M. Taken together, our results enhance the understanding of the virus-host interaction network, and provide a potential target gene as link between apoptosis and cell cycle arrest.

Baculovirus Utilizes Cholesterol Transporter NIEMANN–Pick C1 for Host Cell Entry

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Baculovirus is a powerful tool for biological control in agriculture, and as a tool for foreign gene expression and delivery however, the current understanding of the BmNPV (*B. mori* nuclear polyhedrosis virus) entry mechanism remains limited. Here, we demonstrate that the Bombyx mori Niemann-Pick C1 (BmNPC1) is essential for baculovirus infection in insect cells. Both pretreatment of B. mori embryonic cells (BmE) with NPC1 antagonists and down-regulation of NPC1 expression resulted in a significant reduction in baculovirus BmNPV infectivity. Disruption of BmNPC1 decreased viral entry rather than reduce the viral binding to the BmE cells. Furthermore, our results showed that NPC1 domain C binds directly and specifically to the viral glycoprotein GP64, which is responsible for both receptor binding and fusion. Antibody blocking assay also revealed that the domain C specific polyclonal antibody inhibited BmNPV infection. Purified NPC1-C protein binds to GP64 inhibited virus infection. BmNPC1 confers susceptibility to BmNPV infection when expressed in non-permissive sf9 cells. Our findings support a model Baculovirus binds to its intracellular receptor NPC1, and GP64–NPC1 engagement within lysosomes promotes a late step in entry proximal to viral escape into the host cytoplasm. These results combined with previous studies identifying an essential role of human NPC1 (hNPC1) in filovirus/HAV infection, provide new insights for viruses hijacking cholesterol intracellular transport pathway proteins to infect cells.

HOST PATHOGEN INTERACTIONS
VIDEOS

MicroRNA targeting of Sindbis virus confirms the importance of midgut replication in disseminated infection of *Aedes aegypti*

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In order to develop improved arbovirus control strategies, it is important to better understand how these viruses infect their mosquito vectors. Mosquitoes are typically exposed to arboviruses when they feed on virus-containing blood. Upon ingestion of virus, the first potential target tissue is the mosquito midgut epithelium. However, to be transmitted an arbovirus must escape from the midgut, infect the salivary glands, and be released in the saliva. Arboviruses replicate in midgut cells, but it is unclear whether replication in the midgut is required for midgut escape. We examined this question in Aedes aegypti using microRNA (miRNA) targeting to specifically reduce the ability of Sindbis virus (SINV) to replicate in midgut cells. Sequences with complementarity to two midgut-specific miRNAs were inserted into the SINV genome. Replication of miRNA-targeted viruses was reduced relative to control viruses in cultured cells transfected with the midgut-specific miRNAs. Mosquitoes that were fed the miRNA-targeted viruses were significantly less likely to develop disseminated infection than mosquitoes that were fed control viruses. There was no observed selection for mutations in the miRNA binding sites of virus populations in mosquitoes that developed disseminated infection. Interestingly, in the minority of mosquitoes that developed infection with the miRNA-targeted viruses, the titer of virus in both the midgut and the carcass was not significantly different from mosquitoes infected with control viruses. Thus, if midgut infection was successfully established, midgut replication and dissemination occurred normally. These results indicate that replication in midgut is important for disseminated infection by SINV in *Ae. aegypti*.

Genomic analysis of Oryctes rhinoceros nudivirus (OrNV) and its host, Coconut Rhinoceros Beetle (*Oryctes rhinoceros*), in South Pacific Islands

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Recently, incursions of the Coconut rhinoceros beetle (CRB), have been detected in some south Pacific countries. It has been suggested that this range expansion is related to an *O. rhinoceros* haplotype (CRB-G) that is reported to show reduced susceptibility to the classical biocontrol agent, *Oryctes rhinoceros* nudivirus (OrNV). We previoulsy sequenced the full genome of OrNV from an individual CRB-G haplotype in Solomon Islands. Here, we investigated O. rhinoceros population genetics, OrNV prevalance, the transcription profile of the virus and genomic variations of the virus in CRB specimens collected from across the Pacific. Based on the sequence of the mitochondrial *CoxI* gene, we found three major mitochondrial haplotype groups (CRB-G, CRB-PNG and CRB-S) across the region. The host haplotype diversity varied between and within countries and a high incidence of OrNV infection was detected in all haplotypes wherever they occurred. Genotyping-by-Sequencing (GBS) showed genetic differentiation in the *O. rhinoceros* nuclear genome across populations and provided evidence for gene flow and admixture. The current *CoxI* based method is not a reliable diagnostic marker for phenotypic traits, especially in countries such as Solomon Islands where the mitochondrial haplotypes have come back into sympatry and are mixed. We detected several polymorphic sites in 892 positions of the viral genome. Non-synonymous mutations were detected in several hypothetical proteins, and 15 nudivirus core genes. The phylogenic analysis indicated that OrNV isolates from the Solomon Islands and the Philippines are closely related to each other while isolates from PNG and Fiji form a distinct adjacent clade.

Identification of a PGRP-lb gene in *Spodoptera exigua* with antiviral function against *S. exigua multiple nucleopolyhedrovirus* (SeMNPV)

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In insects, imd pathway is involved in antiviral immune responses. Peptidoglycan recognition proteins (PGRPs) can activate imd immune signaling pathway through the recognition of specific peptidoglycans. However, the function of *PGRP-lb* is unclear in *Spodoptera exigua*. Here, we cloned and identified *PGRP-lb* from *S. exigua*, which consists of five exons that encodes a polypeptide of 234 amino acids with a signal peptide and an extracellular PGRP domain. Our results showed that *S. exigua multiple nucleopolyhedrovirus* (SeMNPV) infection induced up-regulation of *PGRP-lb* expression in *S. exigua* cells. Furthermore, up-regulation of *PGRP-lb* significantly reduced the infection rate of SeMNPV, and inhibited SeMNPV multiplication (reduced the production of polyhedrons and the titer of budding virus). This is the first report of *PGRP-lb* which revealed an antiviral effect in *S. exigua* and provides insights into the function of *PGRP-lb*, a potential anti-SeMNPV mechanism, and a possible target for the controlling of *S. exigua*.

Key words: antiviral; *PGRP-lb*; SeMNPV; *Spodoptera exigua*

Baculovirus infection alters olfaction of its lepidopteran host Spodoptera exigua (Hübner, 1808).

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Olfaction plays an important role in the ability of the insect to perceive the surrounding environment, and it contributes to reproductive tasks, food source detection and enemy avoidance. Baculoviruses are entomopathogenic viruses infecting predominantly insects of the order Lepidoptera, which display extended behavioral phenotypes upon infection. We hypothesize that baculovirus infection may alter the expression of genes controlling the insect chemoperception. In this work, we describe that *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) alters specific gene expression of components of the peripheral nervous system in charge of the insect's olfaction, as the odorant receptors (ORs), when infecting the lepidopteran pest Spodoptera exigua (Lepidoptera: Noctuidae). Certain transcripts encoding ORs were found up-regulated after the infection. One of them, SexiOR35, was selected for further functional characterization by heterologous expression in empty neurons of *Drosophila melanogaster* coupled to single-sensillum recordings, using a panel of 58 stimuli. SexiOR35 resulted in a broadly tuned receptor, able to recognise many different chemical compounds. Behavioral experiments revealed that larvae infected by SeMNPV exhibit altered olfactory-driven behavior to diet when supplemented with the plant volatiles linalool or estragole, two of the main SexiOR35 ligands. This is the first report of baculovirus-associated behavioral changes that are likely linked to alteration in chemoperception through changes in the expression level of specific odorant receptors.

A silent killer of crickets: insights on the transmission of Acheta domesticus densovirus

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The European house cricket, Acheta domesticus is a cosmopolitan cricket species that has long been used for research purposes and has also been mass-reared as reptile pets' food. Currently, there is a global interest in the production of (house) crickets as an alternative source of protein in the growing edible insects industry. In many cases of recent disease outbreaks in large-scale cricket rearing systems, the pathogen infecting the crickets is the Acheta domesticus densovirus (AdDV). AdDV is known to be a devastating pathogen that causes economic loses and that has affected cricket rearing companies in North America and in Europe over the last decade. In our recent research, we hypothesized the global incidence of AdDV to be due to its covert nature. We activated AdDV from the covert to the overt state in crickets from our permanent rearing at WUR. The presence of AdDV in the hemolymph of all examined healthy looking adults (females and males) was tested positive by qualitative PCR. AdDV was also detected in surface-sterilized eggs. We will discuss our insights on AdDV transmission mechanisms and their implications on viral outbreaks in cricket rearing systems.

Investigating the vertical transmission of covert infections by SeMNPV in *Spodoptera exigua*

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Covert viral infections in insects imply the direct transmission of the virus from the infected parents to their offspring while no clear symptoms of disease are shown. Nevertheless, any change in the surrounding environment may cause a stress in the host population and eventually this may lead to the reactivation of the overt state of the infection. Because of the severe economic consequences of sudden disease outbreaks in the emerging field of insect mass rearing, covert infections in insect species are currently of great concern. However, how covert viruses are transmitted to the next generations and how they are maintained in their insect hosts is still unknown. Using Next Generation Sequencing together with additional molecular biology tools we studied the maintenance and transmission of a covert infection of Spodoptera exigua multiple nucleopolyhedrovirus (SeMNPV) in the beet armyworm S. exigua. Ultimately, our results may contribute to the prevention and control of sudden disease outbreaks in insect mass rearing resulting from covert virus infections.

Gene expression profiles of different Cydia pomonella granulovirus isolates in midguts of type II resistant coding moth larvae

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Since the late 1980s, various isolates of the Cydia pomonella granulovirus (CpGV) have been worldwide used as insect pest control agents against codling moth (CM, Cydia pomonella L.), a main pest in apple orchards. Three types (I–III) of dominantly inherited resistance of CM larvae to CpGV have been recently identified. In this study, the midgut transcriptomes of different CpGV isolates, namely CpGV-M, -S, -E2 and -B, breaking or not breaking type II resistance in the CM strain CpR5M were compared at 72 hours post infection by strand-specific RNA sequencing (RNAseq). Principal component analysis of read counts and SNP distribution in the RNAseq data were used for a posteriori identification of the infective agents. The novel isolate CpGV-B was similar or more effective than CpGV-E2 and its transcription level was also higher than that of CpGV-E2. In addition, there was no substantial difference between CpGV-M and CpGV-S transcription found in CpR5M, although a midgut related resistance factor was proposed for CpGV-S. These results indicated that CpGV-S can enter midgut cells. The observed transcription profiles propose highly reduced transcription levels for CpGV-M and CpGV-S compared to CpGV-E2 and CpGV-B.

Key words: CpGV, Cydia pomonella, resistance, transcriptome, viral isolates

HOST PATHOGEN INTERACTIONS
POSTERS

Electron microscopy study confirms infection of coconut rhinoceros beetle (CRB-G) gut cells by OrNV V23B.

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Coconut rhinoceros beetle (Oryctes rhinoceros) (CRB) was considered one of the worst invasive pests in the Pacific until it was brought under control by release of a biocontrol virus *Oryctes nudivirus* (OrNV) in the 20th Century. Recently, a new variant of CRB has invaded several Pacific islands and states. The new variant, CRB-G, is currently causing significant damage to palms and appears to be resistant/tolerant to OrNV isolates that have successfully been used to manage other CRB variants already established within the Pacific. To counter the pest, we have tested a range of OrNV isolates with one isolate (V23B) producing significant mortality in bioassays. DNA extraction and PCR with specific primers confirmed the presence of OrNV in infected beetles. We further examined early stages of OrNV V23B infected beetles using histopathology and light and electron microscopy. The light microscope sections show the swelling of the midgut and the production and release of vesicles and nuclei into the lumen, typical of infection. Electron microscopy revealed high numbers of virions in the swollen vesicles. The results confirm that death of the infected beetles is due to virus infection and provide evidence that the apparent resistance/tolerance of CRB-G to OrNV can be overcome by appropriate strain selection.

ENDOGENOUS VIRUSES
VIDEOS

Characterization of a new nudiviral endogenization event in the Campopleginae wasp *Campoplex capitator*

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Nudiviruses are large double stranded DNA viruses closely related to Baculoviruses. Certain nudiviruses are known to be endogenized in parasitoid wasp species and several independent viral endogenization events have been documented. This wasp-virus symbiosis is advantageous for both protagonists : the wasps make use of viral integrations to successfully accomplish their parasitic life cycle in lepidopteran hosts. Indeed, particles of viral origin are injected in the host during wasp oviposition and protect the wasp progeny by altering the lepidopteran host immune system.

The alphanudivirus endogenized in *Venturia canescens* was the first nudivirus integration reported to this date in ichneumonid wasps. Recently, we characterized an endogenized nudivirus in *Campoplex* capitator, an ichneumonid wasp phylogenetically closely related to *Venturia canescens*. *Campoplex* capitator genomic and transcriptomic analyses revealed that nudivirus sequences are integrated in the wasp genome and expressed in the wasp tissues (ovaries). Electronic microscopy of ovarian calyx cells revealed that the wasp produces Virus-Like-Particles (VLPs) similar to those described in *Venturia canescens*. Therefore, viral elements from *V. canescens* and *C. Capitator* probably originated from the same viral integration event in the genome of a common ancestor of these wasps. This finding offers the opportunity to perform genomic comparisons to better understand mechanisms involved in viral domestications and to determine if the same evolutionary paths were taken to produce VLPs in the two species.

A viral mutualist employs post-hatch transmission for vertical and horizontal spread among parasitoid wasps

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Bacterial symbionts display a wide variety of transmission strategies to travel from one generation of insects to the next. Parasitoid wasps, however, are better known for their heritable associations with viruses, which are highly beneficial for wasp survival during their development as parasites of other insects. Most of these beneficial viral elements are strictly transmitted through the wasp germline as integrated proviruses within wasp genomes. However, a beneficial poxvirus inherited by *Diachasmimorpha longicaudata* wasps, known as *Diachasmimorpha longicaudata* entomopoxvirus (DlEPV), is not integrated into the wasp genome and therefore, may employ different transmission routes to infect future generations of wasps. Here, we uncovered the first documented case of post-hatch transmission for a mutualistic virus, which entails external acquisition and localization of the virus within the adult wasp venom gland. We showed that this route is highly effective for vertical and horizontal transmission of the virus within *D. longicaudata* wasps. Furthermore, the highly beneficial phenotype provided by DlEPV during parasitism was also transmitted with extremely high efficiency, indicating an effective mode of symbiont spread to the advantage of infected wasps. These results provide novel insight into how beneficial viruses can be transmitted and spread among insects.

Endogenous viral element-derived Piwi-Interacting RNAs (piRNAs): insights from Spodoptera genus

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Sequence specific RNA break down activated by small interfering RNAs (siRNA) is considered the main antiviral defense in insects. The Piwi-interacting RNA (piRNA) derived from endogenous viral elements (EVEs) inserted into the host genome has been recently proposed as a complementary antiviral mechanism in Aedes species, where it represents an immune memory against previous RNA virus infections. Whether piRNA-triggered antiviral immunity is present in Lepidoptera is unknown, although several RNA viruses infecting this order have been described. Our study focuses on the annotation of the EVEs present in the genomes of Spodoptera species whose assembly is publicly available. We found that the presence of a handful of EVEs mainly derived from rhabdovirus and orthomyxoviruses is recurrent in the three species studied. We sequenced small RNA from S. exigua and S. frugiperda and the results pinpoint that half of the EVEs present in the genomes of these species does produce piRNAs. We next move to study the molecular evolution of the Argonaute proteins that play a central role in the piRNA biogenesis (AGO3 and SIWI). Our results, based on a phylogenetical framework composed of ten Noctuidae species, suggest a dynamic pattern of Argonaute evolution in this family and concretely, show that Spodoptera genus is characterized by two gene gains. Overall, these results open new avenues to study the so far understudied EVE-derived piRNA immunity in Lepidoptera.

The fusion of envelopes of Microplitis bicoloratus bracovirus during assembly and invasion

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Parasitic systems are established when organisms require special living habits at the level of nutrients between species. Some microorganisms play the important role in suppressing the immune response of the host. Polydnaviruses, which are assembled in the ovary of Hymenoptera wasps and are injected into Lepidoptera insects, are classic insect viruses for explaining the virus envelopes fusion phenomenon. Polydnaviruses are divided into two genera called *Bracovirus* (BVs) and Ichnovirus (IVs). It is unknown how Bracovirus envelopes maintain the dynamic of multiple nucleocapsids during assembly. Here, we utilize transmission electron microscopy to show the ultrastructures of calyx cells of *Microplitis bicoloratus* and High Five cells invasion by Microplitis bicoloratus bracovirus (MbBV). In calyx cells, the assembly process is closely related to nuclear morphology. A single envelope with a nucleocapsid present in the virus assembly cores; around these cores, multiple envelopes fusion to transform a virus envelope with multiple nucleocapsids. This phenomenon suggested that envelopes fusion follows a systematized pattern. Interestingly, in the invasion, High Five cells infected by MbBV, same envelopes fusion are observed nearby plasma membrane, and the fusion of envelopes and plasma membrane of High Five cells release nucleocapsids into the cells. These results reveal the formation of multiple nucleocapsids in an envelope. The better interpret the reason why polydnaviruses are considered as "poly".

Organization and evolution of endogenous bracovirus in parasitoid wasp genomes

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Bracoviruses are essential for the parasitism success of parasitoid wasps, into whose genomes they integrated ~103 million years ago. Their study offers clues to better understand how large DNA viruses integrated into a genome evolve when they confer a benefit to their host. From the assembly of a parasitoid wasp genome at a chromosomal scale, we showed that bracovirus genes colonized all ten chromosomes of the parasitoid wasp *Cotesia congregata*. Most form clusters of genes involved in particle production or parasitism success. Genomic comparison with another wasp, *Microplitis demolitor*, revealed that these clusters were already established ~53 mya and thus belong to remarkably stable genomic structures, the architectures of which are evolutionary constrained. Transcriptomic analyses highlight temporal synchronization of viral gene expression without resulting in immune gene induction, suggesting that no conflicts remain between ancient symbiotic partners when benefits to them converge.

Role of endogenized *lef-4* and *lef-8* nudiviral genes in Virus-Like-Particle production in the parasitoid wasp *Venturia canescens*

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Nudiviruses that are large dsDNA viruses related to baculoviruses, are known to be endogenized in the genomes of certain wasp species. This wasp-virus symbiosis is advantageous for both protagonists, the wasp uses the virus to ensure its reproductive success and the virus is transmitted vertically within the wasp genome. *Venturia canescens*, an ichneumonid wasp belonging to the Campopleginae subfamily, has been shown to have endogenized nudivirus genes belonging to the Alphanudivirus genus. *Venturia canescens* wasps produce Virus-Like-Particules (VcVLPs) which package proteic virulence factors. The aim of this study is to determine whether Alphanudivirus gene functions have been conserved following endogenization. Virus expression within the wasp was investigated by RNA-seq analyses and RNA interference (RNAi) was used to understand the functional role of virus *lef* genes, supposedly encoding the viral RNA polymerase. This study suggests that a transcriptional cascade exists as it is described in baculoviruses and that RNAi is efficient to suppress virus gene expression. Indeed, here we show that alphanudiviral lef genes are expressed at early stages of wasp development and are necessary to transcribe viral genes encoding envelope components. Without the expression of *lef* genes, PIF (*per os* infectivity factors) envelop proteins cannot be produced and VcVLP production is altered as shown by electron microscopy. These results reveal that essential Alphanudivirus functions are conserved after endogenization enabling the production of virus-derived particles that are necessary for wasp development.

Effect of Viral RNA Polymerase on Expression of Wasp and Viral Genes in *Microplitis demolitor*

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The parasitoid wasp, *Microplitis demolitor*, has a unique life cycle of depositing eggs and viral particles into a larval host before consuming the host and pupating. To combat the host's immune response, the wasp produces *Bracovirus* in its ovaries. The *M. demolitor* genome contains virus-derived genes, some of which are expressed in early wasp ovary development and are responsible for encoding a viral RNA polymerase, including subunits encoded by *lef-4* and *lef-9*. In baculoviruses, the viral RNA polymerase specifically transcribes "late" genes, which are expressed after "early" genes and encode structural virus proteins. It is hypothesized that the viral RNA polymerase function is conserved in bracoviruses and involved in transcription of late genes. Previous support for this hypothesis was provided by quantitative PCR, demonstrating that RNAi knockdowns of *lef-4* and *lef-9* genes reduced transcription of two late genes in *M. demolitor*. The objective of this study is to characterize genes transcribed by viral RNA polymerase through RNA-Seq analysis of datasets generated from normal adult ovaries when compared to *lef-4* knockdowns. Results show that when *lef-4* is knocked down, the transcription of 47 out of 62 late genes is downregulated compared to control samples, suggesting they are likely controlled by the viral RNA polymerase. Out of all 12,761 genes analyzed, 243 were shown to have differential expression after *lef-4* knockdown. This study shows that the viral RNA polymerase plays a major role in transcriptional control of the expression of genes involved in *Bracovirus* production in wasp ovaries.

ENDOGENOUS VIRUSES
POSTERS

Induction of apoptosis in insect cells by tyrosine phosphatases from *Cotesia flavipes* bracovirus

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Bracoviruses are a genus of segmented dsDNA viruses that are symbiotically associated with the braconidae family of parasitoid wasps. The Cotesia flavipes wasp is widely used as a biological control agent of the Diatraea saccharalis larvae in sugar crops. During oviposition there is a release of a large amount of viral particles into the caterpillar body that will infect the host hemocytes. This leads to an induced immunosuppression in the host which increases fitness of the wasp`s progenies. In this work, we constructed plasmids containing several Protein Tyrosine Phosphatase (ptp) viral genes derived from CfBV genome for transient expression in insect cells. The plasmids were transfected into Trichoplusia ni cells (Tn5B) and we checked at 48 hours post transfection for morphological changes and effector caspase activity. Cells undergoing apoptosis were observed in transfections with ptp-a, ptp-o, ptp-omega, ptp-q, ptp-w, ptp-n and ptp-t genes. Effector caspase activities were varied but positive compared to negative controls. These results demonstrate that the isolated PTP proteins are sufficient to induce apoptosis in vitro. The functional redundancy within this set of genes correlates well with the presence of conserved phosphatase domains and topologies but in contrast the low overall identities may point to distinct mechanisms of apoptosis induction by each protein which requires in depth assessments.

VIRUS DETECTION AND IDENTIFICATION
VIDEOS

The RNA virome of the medfly: a necessary step to optimize medfly control

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Insect specific viruses (ISVs) are known to specifically infect insect hosts causing no apparent effects on their fitness. The discovery of ISVs has largely increased during recent decades thanks to Next Generation Sequencing techniques, especially in insects with industrial interest. For instance, some RNA viruses were recently discovered in the agricultural pest *Ceratitis capitata*, also known as the Mediterranean fruit fly (medfly). Medfly infestations are mainly counteracted by the sterile insect technique (SIT), which requires the production of tons of sterile medfly males. Mass-rearing facilities offer a controlled way to rear large quantities of insects for different purposes. However, such mass-reared insects are threatened by viral diseases, coming from viruses introduced via horizontal transmission or from already present covert infections that are triggered into overt infections.

Understanding the interaction between covert RNA viruses and the medfly host requires the description and characterization of the RNA virome of the fly host. To do so, we have applied bioinformatics approaches to discover RNA viruses in the transcriptomes of four different medfly populations originating from multiple captures in the citriculture area in Spain. The obtained medfly virome is composed of twelve RNA viruses, five of which are new to medflies. Nine of the detected viruses belong to six known virus families while three are unclassified RNA viruses. Finally, we have defined the genomic structure, the phylogenetic classification, and the relative abundance of the discovered covert RNA viruses in four different laboratory and wild populations of *C. capitata*, including the V8A population used for SIT applications.

Harnessing the Potential of Real Time Portable Next Generation Sequencing as a Surveillance Tool for Pathogens in Mass Reared Insects

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With the rapid expansion of industrial insect rearing facilities, reliable detection of pathogens in insect colonies is essential to prevent or control an infectious disease that could cause significant economic loss. Common diagnostic methods often require prior knowledge of infection symptoms and are restricted to detection of suspected pathogens. The use of metagenomics approaches would allow unbiased identification of organisms in an insect sample, which may be also advantageous for detecting unsuspected pathogens. Here, we evaluated the utility of the portable nanopore-sequencing device, Oxford Nanopore Technology MinION, in combination with the Nvidia Jetson AGX Xavier Developer Kit, as a new tool for the rapid and timely detection of pathogens in insects.

A specifically adapted bioinformatics analysis pipeline was first evaluated on publically available datasets and microbial standards consisting of various microbial communities. Next, the pipeline was evaluated for its potential to detect pathogens using MinION-generated metagenomics datasets of mass reared silkworms (*Bombyx mori*), house crickets (*Acheta domesticus*) and codling moth larvae (*Cydia pomonella*). By linking nanopore sequencing, GPU-based accelerated base calling and the implementation of bioinformatics pipelines on portable device, a rapid and effective solution can be achieved for future routine pathogen surveillance in mass rearing facilities, to maintain healthy insect colonies and preventing economic damage to this emerging industry.

Insect iridescent virus type 6 is widespread in wild and cultured insects

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Insect iridescent virus type 6 (IIV-6) is an insect pathogen with a broad host range including *Orthoptera, Blattodea, Lepidoptera, Coleoptera* and *Diptera*. It is a problem for edible cricketfarming. Search for uninfected colonies is necessary for establishment of healthy cultures. Primers specific to the major capsid protein (mcp) of IIV-6 were used to detect viral infection in insects from Russia, Latvia, Poland, Germany, Uganda and Thailand. None of the cricket colonies was be free from the virus, with its prevalence levels ranging from 33 to 100 %. Partial sequence of the mcp gene was identical in all IIV-6 isolates from hemimetabolous insects, suggesting widespread distribution of a single viral genotype in *Acheta domestica* and *Gryllus bimaculatus* held in captivity in Russia and Thailand, as well as in cultured *Acheta sigillata, Gryllus locorojo, Locusta migratoria, Shelfordella tartarica* and synantropic *Blatella germanica* and *Drosophila melanogaster*. Other PCR-positive cultured insect species were *Schistocerca gregaria, Galleria mellonella, Tenebrio molitor* and *Zophobas morio. Moreover, A. domestica, L. migratoria* and *Pieris brassicae* collected under field conditions in Russia were also virus-positive. Thus, the infection seems to be ubiquitous and finding a virus-free colony can be problematic, even when independent distant sources and field samplings are exploited. Supported by RFBR, project # 19-016-00180.

Insect and plant virus diversity associated with the vine mealybug *Planococcus ficus*

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The grapevine, *Vitis vinifera L.*, is the most worldwide-spread species cultivated for grape production in Europe, Africa, and America. In Mexico, Baja California (B.C.) is the region with the highest grape production for industrial purposes. The vine mealybug, *Planococcus ficus* (Signoret, 1875), is the most important insect pest in grapevine growing areas in Mexico and other countries. Recently, the diversity of viruses infecting insects has been broadly explored to elucidate further ecological viral-host interactions in many insect species, which in some cases has resulted in the application of virus-based biological control agents for insect pests. However, a survey of viruses associated with *P. ficus* has not been done yet. In the present study, *P. ficus* individuals collected through different vineyards of Ensenada, B.C., were analyzed by transcriptomics. A diversity of sequences of putative RNA viruses were found, including a nearly complete genome of a tentatively new member of the Dicistroviridae family and a nearly complete genome of a new isolate related to the Tymoviridae family. Moreover, several smaller, novel virus-like sequences related to the *Tymoviridae, Reoviridae, Tombusviridae, Iflaviridae* families, and the Picornavirales order were identified. Phylogenetic analyses showed that our sequences are related to viruses with hemipteran or insect hosts or vectors, suggesting that some of these putative RNA viruses could specifically infect *P. ficus*. This work is the first insight into the *P. ficus* virome; it guarantees further studies aimed to characterize those viruses potentially infecting P. ficus, in addition to grapevine viruses associated with this mealybug.

Compatibility of covert infections with RNA viruses with natural enemies in *Spodoptera exigua*.

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Research on insect viromes in last years is allowing the fast discovery of novel viral pathogens. Some of these newly discovered viruses produce covert infections that do not cause evident symptoms. In previous works, three RNA viruses producing covert infections on the beet armyworm, *Spodoptera exigua*, were discovered. These viruses were detected in laboratory as well as field populations, but the viral incidence in field samples was lower than in laboratory, suggesting that covert infections may have sublethal effects on *S. exigua* in nature. In this work, we have analyzed the possible impact of those viral infections on the activity of different natural enemies used in Integrated Pest Management (IPM) programs for the control of *S. exigua*. Specifically, we have studied the effects of *Spodoptera exigua* iflavirus 1 (SeIV1) on the mortality produced by entomopathogenic bacteria, nematodes, fungi, and parasitoids. Infections with SeIV1 showed compatibility with the nematode *Steinernema carpocapsae* as the viral infection did not affect to the susceptibility or production of new juveniles. Moreover, they are also compatible with the fungi *Metarhizium brunneum* as mortality was not affected by the viral infection. Interestingly, infections with SeIV1 increase the susceptibility to Bacillus thuringiensis formulates and increase the mortality caused by the parasitoid *Hyposoter didymator*. In summary, our results show that covert infections with SeIV1 are compatible with the four natural enemies tested.

Oryctes rhinoceros nudivirus infections of G-haplotype coconut rhinoceros beetles (*Oryctes rhinoceros*) in Palauan PCR-positive populations

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Since 2007, an invasion by Coconut rhinoceros beetle (CRB), *Oryctes rhinoceros*, has been reported in the Pacific, including Guam, Hawaii, Papua New Guinea and Solomon Islands, where palm trees have been significantly damaged by a particular haplotype (clade I), known as "CRB-G", distinguished by a molecular marker in the mitochondrial gene. In Palau Archipelago, it was reported that CRB-G and another haplotype (clade IV) belonging to the CRB-S cluster coexisted in the field. More than 75% of pheromone trap-captured adults of both of haplotypes were OrNV-positive by PCR in Palau, but no significant difference in OrNV prevalence between the haplotypes was detected. Transmission electron microscopy and RT-PCR of CRB larvae injected with homogenates of trap-captured adults confirmed that the adults contained infectious OrNV particles. However, the OrNV isolates from Palauan beetles exhibited a lower level of viral production and longer larval survival times compared to OrNV isolate X2B, a typical isolate used for biological control of CRB in the Pacific. The full genome sequences of the Palauan and X2B isolates were determined and found to be closely related to each other. These results suggest that CRB adults in Palau are infected with a less virulent virus.

VIRUS DETECTION AND IDENTIFICATION
POSTERS

Preliminary observations of viral presence in a mass rearing crickets used as feed and food.

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Mass farming systems of edible insect are relatively new, and little is known about factors determining the quality and yield of production processes. Achieving this knowledge could be essential to avoid diseases in insects, particularly the viral ones, and therefore guarantee the success of insect breeding.

Thus, we investigated mortality outbreaks occurred in farmed condition insects to establish if viral pathogens could be the etiological cause of the observed diseases. We focused on an insect production system for food and feed where a single insect species, *Acheta domesticus (Orthoptera: Gryllidae)*, was reared. The viral presence was revealed by using negative staining EM methods, which , can permit to identify any virus showing typical morphological patterns (so called "catch-all" technique), even in absence of specific reagents or of a reliable suspect

Our observations led to identify the presence of Iridovirus in alive and dead individuals, and in immature and breeding specimens, but not in eggs. The presence of Iridovirus in immature specimens was associated to small (20-30nm) roundish virions, likely resembling dicistrovirus (CrPV) and/or densovirus (AdDV). By using a specific PCR method, the identification of Iridovirus only was confirmed, whereas the identity of small particles is still pending. These preliminary results confirm that at least two viruses, particularly iridovirus stating the high viral load in dead crickets, are closely related to mass rearing of A. domesticus and to mortality events. Indeed, the use of diagnostic techniques (EM and PCR) could help to develop strategies for the surveillance and identification of viruses that can compromise the production process of insects used for feed and food.

VIRAL BIOINSECTICIDE
VIDEOS

Bio-Insecticidal potential of alphabaculovirus and betabaculovirus mixtures to control the Fall Armyworm *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae)

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Baculoviruses are a large group of viruses pathogenic to arthropods, principally insect from the order Lepidoptera, some of which are pests of agricultural importance as *Spodoptera frugiperda*. In this sense, the ability of one isolate of betabaculovirus of S. frugiperda (VG008 - SpfrGV) to enhance the infectivity of one isolate of multiple alphabaculovirus of *S. frugiperda* (SfCOL - SpfrMNPV) was evaluated against S. frugiperda larvae. Bioassays were performed with mixtures by using different proportions 90%:10% (M1), 95%:5% (M2) and 97.5%:2.5% (M3) of SfCOL:VG008, respectively. All mixtures showed higher insecticidal activity that SfCOL. The mixture M3 showed the highest enhancement of SfCOL reducing 11.4 times the Mean Lethal Concentration and 96 h in the Mean Time to Death. The enhancer activity of proteins derived from VG008 (GVPs) was also evaluated in mixture with SfCOL. The GVPs increased 27% larval mortality caused by SfCOL and damaged the peritrophic membrane of *S. litura* larvae, suggesting that the key point in this enhancing activity is the initial step of the midgut infection. M3 was formulated and evaluated under greenhouse conditions in maize plants using different doses. The highest efficacy was obtained with the highest dose of M3 (8 x 10^{11} OBs/ha), result similar to that obtained using the formulated SfCOL in an approximately two-fold higher dose. The viral mixture M3 was selected as the active ingredient to develop a new biopesticide with improved efficacy to control the fall armyworm, a devastating pest that is rapidly spreading around the world.

Characterization of native Mexican strains of baculovirus with virulence towards *Spodoptera frugiperda* (Lepidoptera:Noctuidae)

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The present work had as objective knowing the phylogenetic relationship and *in vitro* permissiveness of six baculoviruses with activity towards *Spodoptera frugiperda* and protein expression of host cells in response to SfNPV-Fx at 8 hours post-infection (h p.i.), as well as the biological characterization of three baculoviruses strains. The infection of the cell line Sf9 was proven with five SfNPVs and one SfGV isolated from *S. frugiperda*, resulting permissive to the infection of four out of the five SfNPVs tested, while the SfGV was non-infective. The genes *lef-8*, *lef-9* y *polh/granulin* were partially amplified from the five SfNPVs and the SfGV studied; nucleotide changes were identified in *lef-8* y *lef-9* from the SfNPVs and *lef-8*, *lef-9* y *granulin* from SfGV-RV. The phylogenetic analysis showed that the strains SfNPV-Arg and the SfNPV, as well as the SfNPV-Ho and SfNPV, turn out to be closely related, just like the strains SfGV-RV and SfGV; the strain SfNPV-An_2 was phylogenetically more distant than the rest of the SfNPVs. The biological effectiveness of three baculoviruses was evaluated, being the SfNPV-Fx baculovirus which presented the lowest LC_{50} (150 OB/mm^2). In addition to the above, the protein expression of host cells in response to SfNPV-Fx at 8 h p.i. was studied, identifying six proteins by MS-MS. With the characterization from these six baculoviruses strains with activity we established the bases in order to continue with the study of the specific mechanisms, evolution and ecology of the isolated baculoviruses from *S. frugiperda* in the American continent.

Multiple baculovirus infections in codling moth: CpGV-R5 help to CpGV-M cannot be substituted by CrpeNPV

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The most common resistance to Cydia pomonella Granulovirus in codling moth is the type I resistance, that results in a complete arrest of CpGV-Mexican isolate replication in all cells of resistant larvae. This arrest can be overcome by supplying CpGV-R5, a virus isolate able to replicate in such resistant larvae. Sequential ingestion of the two virus genotypes yields different results in function of the order of ingestion. Experiments were carried out with virus doses high enough to ensure about 80% mortality of larvae with CpGV-R5, and equal amounts of CpGV-M. No CpGV-M replication was observed when this virus was provided first, while double infections were detected if CpGV-R5 was supplied prior to CpGV-M. The delay between ingestion of the two viruses (30 to 240 minutes) did not influence the frequency of double infections. Cryptophlebia pestaltica nucleopolyhedrovirus (CrpeNPV) efficiently replicates in codling moth larvae, susceptible or resistant to CpGV-M replication. Mixed infections can be obtained by feeding susceptible larvae with CpGV-M and CrpeNPV or with CpGV-R5 and CrpeNPV, or by feeding resistant larvae with mixtures of CpGV-R5 and CrpeNPV. However, unlike CpGV-R5, CrpeNPV is not able to lift the restriction on CpGV-M replication in resistant larvae, indicating that the mechanism involved in resistance is specific.

Amplicon-based sequence analyses of single nucleotide polymorphisms reveal the genetic structure of LdMNPV field populations

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Mass-outbreaks of gypsy moth (*Lymantria dispar*) leading to defoliation of hardwood trees are a serious threat in forestry in Europe and North America that can have a huge impact on the forest's ecology and economic use. In recent years, forests in South Germany were massively infested by gypsy moth. At the same time, natural epizootics with Lymantria dispar multiple nucleopolyhedrovirus (LdMNPV) were frequently noted at different locations. Infected larvae succumb from LdMNPV infections and therefore LdMNPV is of continued interest for the control of gypsy moth populations. To gain insight into the genetic diversity and population structure of naturally occurring LdMNPV infections, we developed novel genome-based analysis tools by using the profiles of single nucleotide polymorphisms (SNPs) in LdMNPV isolated from field-collected gypsy moth larvae from Germany. Next generation genome sequencing combined with SNP analysis revealed different viral genotypes present in natural LdMNPV populations from different sampling locations. Instead of considering the entire LdMNPV genome for further geographic isolate differentiation, a set of 15 selected genomic PCR markers was selected reflecting the genetic variability represented in the SNP profiles of LdMNPV. By using principal component analysis and hierarchical clustering a final subset of five markers covering about 21 SNP positions was identified. These markers are used to study the LdMNPV genotype composition in single gypsy moth larvae by PCR amplicon sequencing.

Resistance of Cydia pomonella to all viral isolates used in biological control in Europe

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C.pomonella is a pest of apple and pear present on all continents, against which one of the most effective non-chemical control method is the use of CpGV, a highly specific virus. Since 2004, cases of resistance to the CpGV-M isolate, the only one used since 1992, have been observed. This problem has been overcome by the use of two alternative viral isolates CpGV-R5 and CpGV-V15 which bypass this resistance in Europe. These are actually mixtures of viral genotypes. In 2020, during a resistance monitoring program conducted on 32 wild populations of C.pomonella from European orchards, we detected for the first time populations with multiple resistance to CpGV-M and CpGV-V15. Moreover, 3 populations show resistance to all three isolates, i.e. all isolates used in France to date. The ratio of resistance of these populations to the CpGV-M isolate is over 300, but only around 10 for the two new isolates. This discovery calls into question this alternative control method, and raises the issue of a very rapid evolution of resistance to a bio-pesticide. The sustainability of biopesticides is therefore questioned.

Yeast-baculovirus synergism for the improved control of *Thaumatotibia leucotreta*, an important pest of citrus in Africa

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Thaumatotibia leucotreta is a phytophagous insect endemic to southern Africa. The baculovirus Cryptophlebia leucotreta granulovirus (CrleGV-SA) forms an integral component of an IPM programme for control of *T. leucotreta* and is highly effective. Due to the mutualistic association identified between *Cydia pomonella* and epiphytic yeasts, resulting in a significant increase in larval mortality, we proposed to determine which yeast species occur naturally in the gut of *T. leucotreta* larvae and to examine whether the isolated yeasts in combinations with CrleGV-SA, enhance its effectiveness. Infested Navel oranges were collected from orchards across South Africa. This led to the isolation and identification of six yeast species via PCR amplification and sequencing of ITS region and D1/D2 domain of the LSU. Larval development and attraction assays were conducted with the isolated yeast species, which were shown to accelerate larval development, reduce mortality and attract neonate *T. leucotreta* for feeding. Oviposition preference assays were conducted with adult *T. leucotreta* females on fruit inoculated with and without yeast. Significantly, more eggs were deposited on yeast-inoculated fruit in two-choice tests. Detached fruit bioassays were then performed to determine the optimal yeast:virus ratio and to further enhance yeast/virus formulation through the addition of an adjuvant and surfactant. The optimal yeast concentration to use alongside CrleGV-SA was determined and the inclusion of an adjuvant and surfactant to the formulation greatly enhanced its efficacy. Semi-field trials were initiated with promising preliminary results being obtained. The results obtained provide a platform for further research into the application of a yeast/virus treatment as a novel control and monitoring option for *T. leucotreta*.

VIRAL BIOINSECTICIDE POSTERS

Insecticidal properties of isolates of *Spodoptera frugiperda* multiple nucleopolyhedrovirus (SfMNPV) against corn- and rice-strain *Spodoptera frugiperda* larvae, and genome analysis of selected SfMNPV isolates

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The fall armyworm, *Spodoptera frugiperda*, is a serious pest of maize in the Americas that has recently invaded and spread through sub-Saharan Africa and southeast Asia. Two genetically distinguishable strains of fall armyworm have been identified: the corn-strain, with larvae prevalent on large grasses such as corn and sorghum; and the rice-strain, typically found on small grasses such as rice or bermudagrass. Bioassays were carried out to determine if larvae of the two strains differed in response to infection with thirteen isolates of Spodoptera frugiperda multiple nucleopolyhedrovirus (SfMNPV). In survival time bioassays with corn-strain larvae, fast-killing (LT_{50} < 56 hr p.i.) and slow-killing (LT_{50} > 68 hr p.i.) categories of isolates could be discerned, while LT_{50}s of rice-strain larvae varied more narrowly. Corn-strain larvae differed by up to 14-fold in their susceptibility to different isolates, with slow-killing isolates exhibiting significantly lower LC50 values then fast-killing isolates. Isolates 459 (Colombia) and 1197 (Georgia, USA) exhibited LC50 values with rice-strain larvae that were 4.6- to 10.9-fold lower than that of corn-strain larvae. Genome sequences were determined for fast-killing isolates 459 and 1197 and slow-killing isolate 281 (Georgia, USA). The genomes of these isolates shared a high degree of pairwise sequence identity with other SfMNPV genomes (99.14% - 99.93%), and phylogenetic inference from genome-length alignments revealed a lack of congruence between geographic origins and genetic relationships of fully-sequenced isolates. Isolate 459 contained a region resulting from recombination with a different *Spodoptera alphabaculovirus*, and the same region was identified in other isolates by PCR.

TRANSDISCIPLINARY

INSECTS AS FOOD AND FEED AND MASS REARING
VIDEOSS

Identification and quantification of entomopathogenic viruses in reared crickets

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Interest in developing sustainable protein alternatives for human consumption, both direct and indirect via animal feed, has gained incredible momentum. One such alternative is insect-derived protein and crickets (family: Gryllidae) are an especially popular group of edible insects due to their nutritional quality and palatability. However, this emerging insect crop has been severely impacted by microbial entomopathogenic infections of which we know very little about. Here, I identified and quantified pathogenic viruses isolated from colonies of *Gryllodes sigillatus* and *Acheta domesticus* crickets, using various molecular and genomic techniques. I will discuss these results and highlight the future considerations necessary for ensuring the health of farmed insects.

The RNA virome of the medfly: a necessary step to optimize medfly control

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Insect specific viruses (ISVs) are known to specifically infect insect hosts causing no apparent effects on their fitness. The discovery of ISVs has largely increased during recent decades thanks to Next Generation Sequencing techniques, especially in insects with industrial interest. For instance, some RNA viruses were recently discovered in the agricultural pest *Ceratitis capitata*, also known as the Mediterranean fruit fly (medfly). Medfly infestations are mainly counteracted by the sterile insect technique (SIT), which requires the production of tons of sterile medfly males. Mass-rearing facilities offer a controlled way to rear large quantities of insects for different purposes. However, such mass-reared insects are threatened by viral diseases, coming from viruses introduced via horizontal transmission or from already present covert infections that are triggered into overt infections.

Understanding the interaction between covert RNA viruses and the medfly host requires the description and characterization of the RNA virome of the fly host. To do so, we have applied bioinformatics approaches to discover RNA viruses in the transcriptomes of four different medfly populations originating from multiple captures in the citriculture area in Spain. The obtained medfly virome is composed of twelve RNA viruses, five of which are new to medflies. Nine of the detected viruses belong to six known virus families while three are unclassified RNA viruses. Finally, we have defined the genomic structure, the phylogenetic classification, and the relative abundance of the discovered covert RNA viruses in four different laboratory and wild populations of *C. capitata*, including the V8A population used for SIT applications.

Immune priming in *Tenebrio molitor* induced by temperature stress and a fungal pathogen

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Pathogens spreading in insect populations reared at high densities can cause devastating losses in commercial rearing facilities. An improved understanding of insect immunity and interactions between pathogens and insects under their rearing conditions is therefore a crucial aspect of alleviating such disease outbreaks. Insects have a form of innate immune memory called "immune priming" (IP), which protects them from infections when previously exposed to a sub-lethal dose of a pathogen. Abiotic stressors, such as temperature, can also induce IP and increase resistance of insects exposed to pathogens. IP is only beneficial in environments in which the insects are repeatedly exposed to pathogens and having an increased immune response over a prolonged time can have a negative impact on other host traits, such as growth and development. In this study, the IP effects induced by temperature shocks (heat and cold shock) were compared with the IP effects induced by a sub-lethal dose of the entomopathogenic fungus *Metarhizium brunneum* both alone, and in combination to study potential interactions. Pathogen susceptibility and effect on molting of immune primed larvae were assessed after subsequent exposure to a lethal dose of *M. brunneum*. Additionally, immune responses (phenoloxidase activity, hemocyte concentration and antibacterial activity of the hemolymph) of immune primed larvae were measured, alongside larval weight increase, duration until pupation and pupal weight. We discuss how the effect of abiotic and biotic stressors on immunity and development of insects can be considered when manipulating rearing environments to maintain insects that have an increased resistance to pathogens.

Identification and Tissue tropism of newly identified iflavirus and negevirus in tsetse flies *Glossina morsitans morsitans*

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Tsetse flies cause major health and economic problems as they transmit trypanosomes causing sleeping sickness in humans (Human African Trypanosomosis, HAT) and nagana in animals (African Animal Trypanosomosis, AAT). One solution to control the spread of these flies and HAT is the Sterile Insect Technique (SIT), whose success relies on establishment and maintenance of healthy and competitive insect colonies. However, mass production of tsetse is threatened by covert virus infections, such as the *Glossina pallidipes* salivary gland hypertrophy virus (GpSGHV), a large, rod-shaped dsDNA virus. This virus infection can switch from a covert asymptomatic to an overt symptomatic state, which can cause the collapse of an entire fly-colony. Meanwhile, GpSHGV can be managed reasonably well, but the identification of additional covert viruses poses other viral threats to tsetse colonies. Recently, we demonstrated the presence of an iflavirus and a negevirus, both positive sense, single stranded RNA viruses, in *Glossina morsitans morsitans*. Besides *G. m. morsitans*, other *Glossina* species in the Seibersdorf rearing facility (Insect Pest Control Laboratory, IAEA, Austria) have been shown to carry both viruses. In the current study we assessed the prevalence of the iflavirus and negevirus in different teste fly species. We analyzed the tissue tropism of these viruses in *G. m. morsitans* hosts to decipher their mode of transmission. Our results demonstrate the presence of both viruses in host tissues such as the brain and fat bodies, but also in their reproductive organs (ovaries and testes), milk and salivary glands, suggesting potential viral transmission either horizontally during feeding and/or vertically from parents to offspring. This study provides the first biological data on the potential effects of iflavirus and negevirus on tsetse colonies.

Harnessing the Potential of Real Time Portable Next Generation Sequencing as a Surveillance Tool for Pathogens in Mass Reared Insects

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With the rapid expansion of industrial insect rearing facilities, reliable detection of pathogens in insect colonies is essential to prevent or control an infectious disease that could cause significant economic loss. Common diagnostic methods often require prior knowledge of infection symptoms and are restricted to detection of suspected pathogens. The use of metagenomics approaches would allow unbiased identification of organisms in an insect sample, which may be also advantageous for detecting unsuspected pathogens. Here, we evaluated the utility of the portable nanopore-sequencing device, Oxford Nanopore Technology MinION, in combination with the Nvidia Jetson AGX Xavier Developer Kit, as a new tool for the rapid and timely detection of pathogens in insects.

A specifically adapted bioinformatics analysis pipeline was first evaluated on publically available datasets and microbial standards consisting of various microbial communities. Next, the pipeline was evaluated for its potential to detect pathogens using MinION-generated metagenomics datasets of mass reared silkworms (*Bombyx mori*), house crickets (*Acheta domesticus*) and codling moth larvae (*Cydia pomonella*). By linking nanopore sequencing, GPU-based accelerated base calling and the implementation of bioinformatics pipelines on portable device, a rapid and effective solution can be achieved for future routine pathogen surveillance in mass rearing facilities, to maintain healthy insect colonies and preventing economic damage to this emerging industry.

A silent killer of crickets: insights on the transmission of Acheta domesticus densovirus

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The European house cricket, Acheta domesticus is a cosmopolitan cricket species that has long been used for research purposes and has also been mass-reared as reptile pets' food. Currently, there is a global interest in the production of (house) crickets as an alternative source of protein in the growing edible insects industry. In many cases of recent disease outbreaks in large-scale cricket rearing systems, the pathogen infecting the crickets is the Acheta domesticus densovirus (AdDV). AdDV is known to be a devastating pathogen that causes economic loses and that has affected cricket rearing companies in North America and in Europe over the last decade. In our recent research, we hypothesized the global incidence of AdDV to be due to its covert nature. We activated AdDV from the covert to the overt state in crickets from our permanent rearing at WUR. The presence of AdDV in the hemolymph of all examined healthy looking adults (females and males) was tested positive by qualitative PCR. AdDV was also detected in surface-sterilized eggs. We will discuss our insights on AdDV transmission mechanisms and their implications on viral outbreaks in cricket rearing systems.

Insect iridescent virus type 6 is widespread in wild and cultured insects

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Insect iridescent virus type 6 (IIV-6) is an insect pathogen with a broad host range including *Orthoptera, Blattodea, Lepidoptera, Coleoptera* and *Diptera*. It is a problem for edible cricketfarming. Search for uninfected colonies is necessary for establishment of healthy cultures. Primers specific to the major capsid protein (mcp) of IIV-6 were used to detect viral infection in insects from Russia, Latvia, Poland, Germany, Uganda and Thailand. None of the cricket colonies was be free from the virus, with its prevalence levels ranging from 33 to 100 %. Partial sequence of the mcp gene was identical in all IIV-6 isolates from hemimetabolous insects, suggesting widespread distribution of a single viral genotype in *Acheta domestica* and *Gryllus bimaculatus* held in captivity in Russia and Thailand, as well as in cultured *Acheta sigillata, Gryllus locorojo, Locusta migratoria, Shelfordella* tartarica and synantropic *Blatella germanica* and *Drosophila melanogaster*. Other PCR-positive cultured insect species were *Schistocerca gregaria, Galleria mellonella, Tenebrio molitor* and *Zophobas morio*. Moreover, *A. domestica, L. migratoria* and *Pieris brassicae* collected under field conditions in Russia were also virus-positive. Thus, the infection seems to be ubiquitous and finding a virus-free colony can be problematic, even when independent distant sources and field samplings are exploited. Supported by RFBR, project # 19-016-00180.

First evidence of long-lasting association between viruses and the Black soldier fly, Hermetia illucens

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Black soldier flies (BSF) are regarded as robust insects for their broad diet and are experiencing fast production growth within the insect as feed and food industry. Some BSF farms experience mortality episodes yet very little is known on BSF pathogens, particularly concerning viruses. As traces of contemporary and past viral infections can be mined in transcriptomics and genomic datasets, we undertook a bioinformatic approach to explore publicly available BSF data. Using Virsorter2, CheckV and BLAST on assembled contigs and scaffolds, we uncovered several viral sequences associated with multiple genomic and transcriptomic datasets. In particular, transcriptomic data led to the genome assembly of an uncharacterized virus, that we refer to as "virus T". In parallel, the use of a novel pipeline on three BSF genomes collected in different countries allowed the discovery of multiple candidate endogenous viral element (EVE) sequences. Analysis of the EVEs shared by all three BSF genomes revealed that some EVEs had nearly identical sequences, indicating that their integration in the BSF genome is not recent. Of note, a short sequence that is highly similar to one group of these EVEs was found to be expressed in BSF, suggesting possible antiviral activity. Lastly, sequence comparison revealed that these EVEs are related to, but different from virus T. Altogether, the results suggest that virus T is an exogenous virus producing an active infection, and that related viruses have long been associated with BSFs.

Drivers and role of bacterial diversity and composition along the developmental stages of the Black Soldier Fly (*Hermetia illucens*)

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Gut bacterial communities play a key role in a number of essential functions in the different developmental stages of insects, and the Black Soldier Fly or BSF (*Hermetia illucens*) is no exception. Shedding light onto the gut bacterial diversity and composition of BSF is important for its use in waste conversion and animal feed production, being efficient insects at bioconversion processes. The main aim of this project is to characterize the microbial communities along the four developmental stages of BSF: eggs, larva, pupa and adult. We explored whether there were significant differences between the composition and diversity of the four stages and whether these differences were mainly driven by host (vertical transmission) or by diet (horizontal transmission). Finally, we aimed to infer the functions of the main microbial taxa and their role in the development of BSF and its bioconversion abilities. To this purpose, we carry a 16S metabarcoding analysis on sampling comprising 141 BSF specimens, which covered the four main developmental stages of BSF and coming from four different rearing colonies fed with three different diets to. Our results reveal the presence of a core microbiota shared by all four developmental stages as well as significant variations in microbial communities. These variations seem to be mainly driven by the diet composition, in accordance with previous studies. Several bacterial taxa detected in our study seem to play an important role in certain functions carried out in the different life stages of BSF and could be candidates to improve its industrial production for bioconversion processes.

The role of the microbiota in host resistance to pathogens in *Galleria mellonella* larvae.

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Currently, it is unclear to what extent the gut microbiota contributes to- or decreases host resistance to pathogens; and if there are some indigenous isolates of the microbiota that may improve the immune response to pathogens. *Galleria mellonella* (Lepidoptera: Pyralidae) larvae, an established model organism, will be reared in sterile conditions resulting in the production of axenic larvae without microbiota. By utilising *Bacillus thuringiensis*, and *Metarhizium anisopliae*, the role of the gut microbiota can be assessed via two routes of infection, in both axenic and conventional larvae. We have found that axenic *G. mellonella* larvae were more susceptible to orally administered *B. thuringiensis* than conventionally reared larvae. At a half-lethal dose, surviving conventional larvae can completely clear *B. thuringiensis* from their gut by 96 hours however; in surviving axenic larvae, the number of cells remains high. Our results also indicated that percentage mortality was always greater for the axenic larvae where 100% mortality was reached by 96h as compared to 50% mortality for conventional larvae. To further investigate the partnership between the microbiota and the immune response, several important immune response genes from the gut and fat body will be measured using qRT-PCR techniques and the composition and dynamics of the gut microbiota will be characterised during pathogen infection using 16S rRNA and the Illumina Miseq platform. There will also be an assessment of important life history traits to determine trade-offs corresponding to the presence or absence of the gut microbiota when challenged by a pathogen. These results will provide an understanding of how and to what extent the immune response is mediated by the microbiota, which can improve the potential for the development of probiotics and overall maintenance of insect health.

Silver nanoparticles are effective in controlling microsporidia .

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Many approaches and technologies have been developed as treatments for microsporidian, infections but effective, broad-spectrum and sustainable therapeutic approaches have not been found. Silver nanoparticles (AgNPs) have antimicrobial activity and are widely used against many different pathogens. AgNPs provide an opportunity to develop formulations that will control microsporidia. In this study, we synthesized AgNPs via a chemical reduction method and evaluated their formation, morphology and stability using transmission electron microscopy (TEM) and ultraviolet spectroscopy analysis. AgNPs damaged to the spore cell membrane and disrupted spore germination of microsporidia *Nosema bombycis*. This resulted in the release of microsporidia nucleic acids, proteins and respiratory chain enzymes. The anti-microsporidia activity of AgNPs was studied by measuring the silkworm larvae survival rate and spore genome replication after microsporidia infection. AgNPs have anti-microsporidian activity and could be effective components of formulations for treating or preventing microsporidia infection.

INSECTS AS FOOD AND FEED AND MASS REARING
POSTERS

Preliminary observations of viral presence in a mass rearing crickets used as feed and food.

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Mass farming systems of edible insect are relatively new, and little is known about factors determining the quality and yield of production processes. Achieving this knowledge could be essential to avoid diseases in insects, particularly the viral ones, and therefore guarantee the success of insect breeding.

Thus, we investigated mortality outbreaks occurred in farmed condition insects to establish if viral pathogens could be the etiological cause of the observed diseases. We focused on an insect production system for food and feed where a single insect species, *Acheta domesticus* (*Orthoptera: Gryllidae*), was reared. The viral presence was revealed by using negative staining EM methods, which , can permit to identify any virus showing typical morphological patterns (so called "catch-all" technique), even in absence of specific reagents or of a reliable suspect

Our observations led to identify the presence of Iridovirus in alive and dead individuals, and in immature and breeding specimens, but not in eggs. The presence of Iridovirus in immature specimens was associated to small (20-30nm) roundish virions, likely resembling dicistrovirus (CrPV) and/or densovirus (AdDV). By using a specific PCR method, the identification of Iridovirus only was confirmed, whereas the identity of small particles is still pending. These preliminary results confirm that at least two viruses, particularly iridovirus stating the high viral load in dead crickets, are closely related to mass rearing of *A. domesticus* and to mortality events. Indeed, the use of diagnostic techniques (EM and PCR) could help to develop strategies for the surveillance and identification of viruses that can compromise the production process of insects used for feed and food.

Effect of Diet and Antibiotic on the growth and fitness of laboratory reared *Spodoptera exigua* (Hübner).

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The success of insect mass rearing is largely dependent on an all-round optimal feed substrate. However, the dietary requirements for optimal growth, pupation, moth development, and survival for many laboratory-reared insects, are not well described. Moreover, it has been shown that diet contributes essential gut microbiota for the growth and survival of insect colonies but antibiotics, which are routinely used in mass rearing, have an effect on these. In this study, *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae) was reared on two artificial diets, Alfalfa-based and wheat germ based in the presence and absence of Streptomycin antibiotic. Growth and fitness parameters recorded included: Growth rate (mg/day), larval period, percentage pupation, pupal weight, pupal period, percentage moth emergence and wing malformation and the survival rate. Overall, the colony performed better in Alfalfa based diet in all parameters measured, in two consecutive generations. Growth rate was highest in Alfalfa with antibiotic but this was not significantly different to the antibiotic treated group. In the absence of antibiotic, moths had a longer oviposition period although the pupal period was longer. Interestingly, there was a significantly lower rate of wing malformation in the Alfalfa based diet without antibiotic compared to the antibiotic treated group (3.75% and 32.25% respectively). These results suggest that the Alfalfa based diet likely contributes essential nutrients and key microbiota components for the growth and fitness of *Spodoptera exigua* and a deeper understanding of these interactions would be useful for the insect rearing industry.

Impact of probiotic bacteria on *Tenebrio molitor* fitness, gut microbial composition and susceptibility to *Bacillus thuringiensis serovar tenebrionis* and *Metarhizium brunneum infections*

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Tenebrio molitor (Coleoptera L.) the yellow mealworm is an insect model for infection and immunity studies and is used in mass-production of insects as feed and food. The industrial rearing of *T. molitor* on agricultural by-products may expose them to biocontrol residues, like environmental resistant *Bacillus* spores and fungal conidia, which could impact the fitness of T. molitor. Therefore, my PhD project deals with experiments analyzing different outcomes of single and co-infections of *B. thuringiensis*, and *Metarhizium brunneum* on the larval stages of *T. molitor*. Furthermore, as for other animals, the possible benefits of addition of probiotic bacteria to the feed will be analyzed. The pathogenicity of *B. thuringiensis serovar tenebrionis* (Btt) and *Metahrizium brunneum* KVL 12-30 are first tested by single infection on *T. molitor* to define LD25 and LD50. Then targeted co-infections will allow to determine additive, synergistic or antagonistic interactions between these pathogens. Alongside infections, feed uptake, growth rate etc. are recorded and gut microbiota composition is analyzed by 16s rRNA Mi-sequencing to measure how probiotic and pathogens modify the OTUs' composition. The hypotheses are: 1) *M. brunneum* and *Btt* have different mechanisms of infection, therefore dose and timing of pathogen exposure should influence the outcome 2) the presence of probiotics may help the insect to cope with the infection by improved immunity, by presenting a shorter period for pathogen clearance, by expressing better fitness performances etc. The poster will include the experimental set up and preliminary results.

Quantification of filamentous growth of entomopathogenic fungi using spectrophotometry for rapid and high-throughput analysis

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Quantifying growth is the fundamental basis for many studies involving fungi and for many pathogenic fungi related to virulence and pathogenic potential. However, traditional methods for determining growth involving measuring biomass or colony growth area can be time consuming, which limits large scale, multi-factorial studies. Here we develop a method for rapidly measuring fungal growth in small-volume liquid media cultured in 96-well microplates using spectrophotometry. To verify our measurements of growth, change in absorbance over time is compared to dry weight of samples and colony growth area on agar plates. This allows for correlation of absorbance values to quantified biomass and generation of growth curves. We analyse 9 different isolates of *Metarhizium* spp. with this technique for treatments involving different temperature and nutrition gradients. We aim to develop spectrophotometric analysis of liquid cultures in microplates as an effective, reproducible, and simple method for rapidly measuring filamentous fungal growth.

CONTRIBUTORS

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