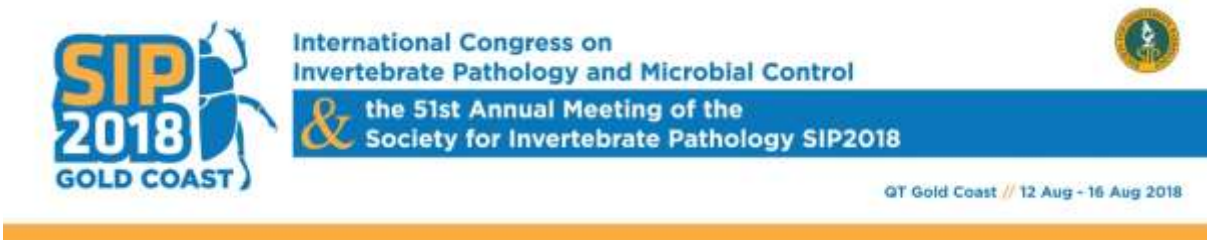
SIP 2018 GOLD COAST

The 2018 International Congress of Invertebrate Pathology and Microbial Control
and the 51st Annual Meeting of the Society for Invertebrate Pathology

QT Gold Coast // Sun 12 Aug - Thu 16 Aug 2018



51st ANNUAL MEETING

of the

SOCIETY FOR INVERTEBRATE PATHOLOGY

and

INTERNATIONAL CONGRESS ON INVERTEBRATE PATHOLOGY AND MICROBIAL CONTROL

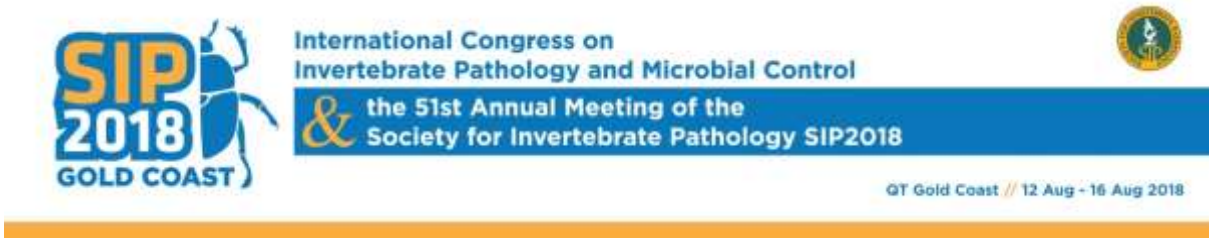
12-16 August 2018

QT GOLD COAST HOTEL

SURFERS PARADISE

QUEENSLAND, AUSTRALIA

At a glance	Programme for SIP2018	
Sunday 12 August 2018		
8:30-17.00	SIP Executive meeting	Malibu
	Registration	Hotel foyer
13.00-17.00	Bacterial Division Workshop: Protein specificity and its impact on safety and resistance	Cloudbreak, Northbreak and Southbreak
17.30 - 19.30	**Welcome Mixer**	Stingray bar
Monday 13 August 2018		
8.00-8.30	Welcome	Pipeline
8.30-10.00	Founders lecture	Pipeline
10-10.30	Morning tea	
10.30-12.30	Plenary Symposium. Insect pathology and microbial control – progress and prospects in the Asia-Pacific region	Pipeline
12.30-1.30	Lunch (lunch is NOT supplied)	
12.30-1.30	JIP meeting	Southbreak
13.30-15.30	Nematode Division Symposium Use of Parasitic Nematodes to Control Pine-Killing Woodwasps	Pipeline
	Fungi Contributed papers 1	Maui 3
	Viruses Contributed papers 1	Maui 1&2
15.30-16.00	Afternoon tea	
16.00-18.00	Microbial Control Division Symposium The challenge of CRB-G to palm production in the Pacific and prospects for microbial control.	Pipeline
	Beneficial Invertrebrates and Microsporidia contributed papers 1	Maui 1&2
18.00-20.00	ICTV Baculoviridae/Nudiviridae Study Group	Southbreak
20.00-22.00	Microbial Control Division business meeting	Maui 2
	Viruse Division business meeting	Maui 3
	Microsporida Division business meeting	Northbreak
	Bacteria Division business meeting	Cloudbreak
Tuesday 14 August 2018		
8.00-10.00	Virus Division Symposium Interactions between arboviruses and their vectors	Pipeline
	Bacteria Contributed papers 1	Maui 1&2
10-10.30	Morning tea	
10.30-12.30	Bacterial Division Symposium Insect resistance mechanisms to Bt.	Pipeline
	Virus Contributed papers 2	Maui 1&2
12.30-17.00	Excursions	
17.00-17.10	BBQ: Buses depart from QT	
19.00-22.00	BBQ dinner	
Wednesday 15 August 2018		
8.00-10.00	Bacterial Division Symposium Insecticidal protein structures	Pipeline
	Microbial Control Contributed papers 1	Maui 1 and 2
	Viruses Contributed papers 3	Maui 3
10-10.30	Morning tea	
10.30-12.30	Diseases of Beneficial Insects Division Symposium Title: Health issues of bee and non-bee pollinators	Pipeline
	Microbial control contributed papers 2	Maui 1&2
12.30-13.30	lunch (lunch is NOT supplied)	
12.30-13.30	Nematode Division business meeting	Maui 3
13.30-15.30	Posters	Conference foyer
15.30-16.00	Afternoon tea	
16.00-18.00	Bacteria Contributed papers 2	Pipeline
	Beneficial Invertrbates and Micosporida continuted papers 2	Maui 3
	Fungi Contributed papers 2	Maui 1 and 2
20.00-22.00	DBI Division business meeting	Maui 3
	Fungi Division business meeting	Maui 1 and 2
Thursday 16 August 2018		
8.00-10.00	Fungal Division Symposium Fungus-insect interactions in post genomic era: Advances and perspectives	Pipeline
	Viruses Contributed papers 4	Maui 1&2
	Microbial control contributed papers 3	Maui 3
10-10.30	Morning tea	
10.30-12.30	SIP General Membership meeting	Pipeline
12.30-1.30	Lunch (lunch is NOT supplied)	
	Science careers workshop	Maui 3
13.30-15.30	Cross Divisional Symposium (Virus and Diseases of Beneficial Insects) White Spot Syndrome Virus - Emergence and control	Pipeline
	Nematodes contributed papers 1	Maui 3
	Bacteria Contributed papers 3	Maui 1& 2
	No sessions	
19.00-	Conference banquet	



51st ANNUAL MEETING

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INTERNATIONAL CONGRESS ON INVERTEBRATE PATHOLOGY AND MICROBIAL CONTROL

PROGRAMME and ABSTRACTS

12 – 16 AUGUST 2018

QT GOLD COAST HOTEL

Surfers Paradise, Australia

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SUNDAY 12 August 2018

9.00-17.00 Registration Hotel Foyer

9.00-17.00 SIP Council Meeting Malibu

13.00-17.00 Workshop Cloudbreak, Northbreak, & Southbreak

Protein specificity and its impact on safety and resistance

17.30-19.30 WELCOME MIXER Stingray Bar

MONDAY 13 August 2018

08.00-08.30 WELCOME Pipeline

08.30-10.00 FOUNDERS LECTURE Pipeline

Ray Akhurst: A legacy of groundbreaking contributions in insect pathology and nematology. *Patricia Stock*

10-10.30 MORNING TEA

10.30-12.30 *Plenary Symposium* Pipeline

Insect Pathology and Microbial Control – progress and prospects in the Asia-Pacific region

Microbial control in New Zealand. *Travis Glare*

Microbial control in pest management and IRMS in Australia. *Caroline Hauxwell*

Progress of *Bacillus thuringiensis* research and application in China. *Ming Sun*

Microbial control as a component of IPM in the production of oil palm in Malaysia. *Norman Kamarudin*

Microbial control for the Pacific Island states. *Sean Marshall*

12.30-13.30 LUNCH (lunch is NOT supplied)

12.30-1.30 JIP MEETING Southbreak

13.30-15.30 Symposium (Nematodes) Pipeline

Use of Parasitic Nematodes to Control Pine-Killing Woodwasps

Control of sirex using the nematode *Beddingia siricidicola*: Past, Present and Future. *Robin Bedding*

Mechanisms responsible for *Sirex noctilio* nematode biocontrol program disruption in Australia. *Angus Carnegie*

Predicting Sirex biocontrol success in subtropical Australia: can *Deladenus siricidicola* take the heat? *Helen Nahrung*

Genetic diversity in global collection of *Deladenus siricidicola. Katrin Fitza*

Potential for non-target effects using biological control nematode against *Sirex noctilio* in North America. *Ann Hajek*

13.30-15.30 FUNGI 1 Maui 3

VIRUS 1 Maui 1 & 2

15.30-16.00 AFTERNOON TEA

16.00-18.00 Symposium (Microbial Control) Pipeline

The challenge of CRB-G to palm production in the Pacific and prospects for microbial control

Progress with control of a virus resistant coconut rhinoceros beetle. *Sean. Marshall*

Attempted microbial control of coconut rhinoceros beetle, *Oryctes rhinoceros*, Biotype G on Guam using *Oryctes rhinoceros* nudivirus and *Metarhizium majus. Aubrey Moore*

Biotype and diversity of *Oryctes rhinoceros* in Japan. *Madoka Nakai*

CRB damage and resistance assessment in the Palau Archipelago. *Madoka Nakai*

Infectivity of Malaysian *Oryctes* nudivirus (OrNV) propagated in insect cell line DSIR-HA-1179 against the rhinoceros beetle, *Oryctes rhinoceros. Nur Ain Farhah Ros Saidon Khudri*

Coconut Rhinoceros Beetle (CRB) control efforts in oil palm: Papua New Guinea (CRB-P) versus Solomon Islands (CRB-G). Mark Ero

The status of Coconut Rhinoceros Beetle, *Oryctes rhinoceros* (L) Scarabaeidae : Dynastinae, in Solomon Islands. *Francis Tsatsia*

Challenge of a new biotype of the coconut rhinoceros beetle (CRB-G) to the Pacific. *Maclean Vaqalo*

16.00-18.00 Beneficials & microsporidia 1 Maui 1 & 2

18.00-20.00 ICTV Nudiviridae Study Group Southbreak

20.00-22.00 Viruses Business meeting Maui 3

20.00-22.00 Microsporida Business meeting Northbreak

20.00-22.00 Bacteria Business meeting Cloudbreak

20.00-22.00 Microbial Control business meeting Maui 2

Tuesday 14 August 2018

08.00-10.00 Symposium (Virus) Pipeline

Interactions between arboviruses and their vectors

Barriers to arbovirus infection in mosquitoes. *Rollie Clem*

Mosquito and viral determinants that condition host specificity, tissue tropisms and transmission: spotlight on the flaviviruses. *Lyric Bartholomay*

Commensal Viruses of Mosquitoes: Host Restriction, Transmission, and Interaction with Arboviral Pathogens. *Jody Hobson-Peters*

Contribution of microRNAs in mosquito-virus interactions. *Sassan Asgari*

8.00-10.00 BACTERIA 1 Maui 1 & 2

10-10.30 MORNING TEA

10.30-12.30 Symposium (Bacteria) Pipeline

Insect resistance mechanisms to Bt.

Combining deleterious ABC transporter C2 alleles of independent origin causes field resistance to insecticidal Bt toxins. *Simon Baxter*

Bt Resistance in Australian Insect Pests. *Sharon Downes*

MAPK signaling pathway *trans*-regulates differential expression of aminopeptidases N and confers resistance to *Bacillus thuringiensis* Cry1Ac toxin in diamondback moth. *Zhaojiang Guo*

Field-evolved resistance to Bt corn in fall armyworm: mechanism, dispersal and biological implications. *Juan Luis Jurat-Fuentes*

Mutations of ABC transporters and Bt resistance in cabbage loopers. *Ping Wang*

Function and role of ATP-binding cassette transporters as a Cry toxins receptor. *Ryoichi Sato*

10.30-12.30 VIRUS 2 Maui 1 & 2
12.30-17.00 EXCURSIONS
17.00-17.10 BUSES DEPART FROM QT
19.00-22.00 BBQ DINNER

WEDNESDAY 15 August 2018

08.00-10.00 Symposium (Bacteria) Pipeline

Insecticidal protein structures

Cryo-EM structure of an insecticidal toxin–ion channel complex reveals thecomplex molecular basis of allosteric modulation of channel gating. *Glenn King*

Pleurotolysin: a pore forming toxin from the carnivorous oyster mushroom. *Michelle Dunstone*

Lessons from the vertebrate immune system: how the membrane attack complex shoots an evolutionary moving target. *Bradley Spicer*

Cryo-EM structures of the pore-forming ABC toxin from *Yersinia entomophaga. Michael Landsberg*

Insights into the cellular recognition patterns of Yentc, an insecticidal pore-forming toxin *Irène Chassagnon*

Insecticidal ABC toxin complexes encapsulate a variety of toxins. *Shaun Lott*

8.00-10.00 MICROBIAL CONTROL 1 Maui 1 & 2
8.00-10.00 VIRUS 3 Maui 3

10.30-12.30 Symposium (Beneficial Insect) Pipeline

Health issues of bee and non-bee pollinators

Viral landscape of Varroa-free Australian honey bees. *John Roberts*

The role of Deformed wing virus (DWV) in Varroa tolerant honey bee populations and its spread beyond bees into the wider insect community. *Laura Brettell*

A natural product inhibited the replication and expression of Israeli acute paralysis virus. *Hou Chunsheng*

Enhancement of chronic bee paralysis virus levels in honeybees acute exposed to imidacloprid: a chinese case study. *Diao Qingyun*

Evidence of deformed wing virus (DWV) – free honey bee populations in the Pacific region. *John Roberts*

10.30-12.30 MICROBIAL CONTROL 2 Maui 1 & 2

12.30-13.30 LUNCH (lunch is NOT supplied)

12.30-13.30 Nematode Division business meeting Maui 3

13.30-15.30 POSTERS CONFERENCE FOYER

15.30-16.00 AFTERNOON TEA

16.00-18.00 BACTERIA 2 Pipeline
16.00-18.00 BENEFICIAL & VIRUSES 2 Maui 3
16.00-18.00 FUNGI 2 Maui 1 & 2

20.00-22.00 DBI Division Business Meeting Maui 1 & 2
20.00-22.00 Fungi Division Business Meeting Maui 3

THURSDAY 16 August 2018

8.00-9.00 Symposium (Fungi) Pipeline

Fungus-insect interactions in post genomic era: Advances and perspectives - Genomic and transcriptomic studies on *Beauveria* including plant associations

Genomic and transcriptomic studies on *Beauveria* including plant associations. *Travis Glare*

Genetic analysis of *Beauveria bassiana* JEF-007 as a biopesticide against bean bug. *Se Jin Lee*

8.00-10.00 VIRUSES 4 Maui 1 & 2
8.00-10.00 MICROBIAL CONTROL 3 Maui 3

10-10.30 MORNING TEA

10.30-12.30 **SIP General Meeting** Pipeline

12.30-13.30 LUNCH (lunch is NOT supplied)

13.30-15.30 Symposium (Cross Divisional) Pipeline

White Spot Syndrome Virus – Emergence and control

Overview of WSSV and its emergence. *Jie Huang*

White spot disease outbreak in farmed prawns in Queensland, Australia in 2016. *Peter Mohr*

Wild type relative of the most important viral pathogen in global aquaculture. *Kelly Bateman*

Potential future therapies for WSSV. *Ornchuma Itsathitphaisarn*

13.30-15.30 NEMATODES 1 Maui 3
13.30-15.30 BACTERIA 3 Maui 1 & 2

19.00 ---CONFERENCE BANQUET

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Meeting Website: Conference OnLine
5K Race: Kristen Knight and Keith Dankwerts
Miscellaneous assistance: Shirley Hauxwell, Andrew Dickson

PROGRAMME 2018

IMPORTANT NOTES:

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

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

SUNDAY 12 August 2018

All Day Hotel Foyer
REGISTRATION

09.00-17.00 Malibu
EXECUTIVE MEETING

Sunday 13.00-17.00 Cloudbreak, Northbreak & Southbreak

Bacterial Division Workshop: Protein specificity and its impact on safety and resistance

Organisers: William Moar and Ken Narva

Most of the Bt "Low hanging fruit" has been picked. Why industry is moving towards non-traditional insecticidal proteins to protect Food Security. Cry51 and beyond.

William Moar, Monsanto Corporation

Update on Bt Nomenclature

Neil Crickmore, University of Sussex, U.K.

Structure/function of the novel coleopteran-toxic protein isolated from *Chromobacterium piscinae*

Jelena Zaitseva, Bayer CropScience

Molecular Lego: Beta pore forming toxin specificity is driven by the ancillary domains

Michelle Dunstone, Monash University, Australia

Structure/function and weight of evidence for specificity of new insecticidal proteins

Lu Liu, Corteva Agriscience™, Agriculture Division of DowDuPont™

Characterization of novel Bt strains against rice plant hoppers

Je Zhang, Chinese Academy of Agricultural Sciences (CAAS), China

Mechanism of Action of Cry34/35Ab1

Ken Narva, Corteva Agriscience™, Agriculture Division of DowDuPont™

Determinants of Vip3A resistance in *Helicoverpa armigera*

Tom Walsh, CSIRO, Australia

Assessing protein interactions between insecticidal proteins

Steve Levine, Monsanto Corporation

Characterization of the Cry1Ah resistance and cross-resistance in Asian corn borer.

Kanglai He, Chinese Academy of Agricultural Sciences (CAAS), China

Assessing the safety of new insecticidal proteins against non-target organisms

Steve Levine, Monsanto Corporation

Evaluating new proteins from a Regulatory Perspective

Dylan Levac, CFIA, Canada

Safety assessment of novel insecticide proteins in GM food crops

Sasha Tait, FSANZ AUS/NZ

Delivery of RNAi by modified chloroplasts for insect protection

Peter Waterhouse, Queensland Univ. of Tech., Australia

17.30-19.30 Stingray Bar
WELCOME MIXER

MONDAY 13 August 2018

08.00-08.30 Pipeline

WELCOME

08.30-10.00 Pipeline

FOUNDERS LECTURE

Honoree: Ray Akhurst

Lecturer: Patricia Stock

Ray Akhurst: A legacy of groundbreaking contributions in insect pathology and nematology

10-10.30 MORNING TEA

Plenary Symposium
Monday 10.30-12.30 Pipeline

Insect Pathology and Microbial Control – progress and prospects in the Asia-Pacific region

Organisers/Moderators: Trevor Jackson and Caroline Hauxwell

10.30 **1** **Microbial control in New Zealand**
Travis R. Glare[1], Maureen O'Callaghan[2]
[1]Bioprotection Research Centre, Lincoln University, Lincoln, New Zealand; [2] AgResearch, Lincoln New Zealand

10.50 **2** **Microbial control in pest management and IRMS in Australia**.
Caroline Hauxwell, Queensland University of Technology, Invertebrate Microbiology Group, Science and Engineering Faculty, Gardens Point Campus, QLD 4001

11.15 **3** **Progress of *Bacillus thuringiensis* research and application in China**
Ming Sun, State Key Laboratory of Agricultural Microbiology, College of Life Science and Technology, Huazhong Agricultural University, Wuhan, Hubei 430070, China

11.40 **4** **Microbial control as a component of IPM in the production of oil palm in Malaysia**
Norman Kamarudin, Mohd Mazmira Mohd Masri and Idris Abu Seman Malaysian Palm Oil Board, No 6, Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia

12.05 **5** **Microbial control for the Pacific Island states**
Sean D.G. Marshall and Trevor A. Jackson, Forage Science, AgResearch, Lincoln Research Centre, Christchurch, New Zealand

12.30-13.30 LUNCH (NOT supplied)

12.30-1.30 Southbreak

JIP MEETING

Nematode Division Symposium –
Monday 13.30-15.30 Pipeline

Use of Parasitic Nematodes to Control Pine-Killing Woodwasps

Organisers/Moderators: Ann Hajek and Helen Nahrung

13.30 **6** **Control of sirex using the nematode *Beddingia siricidicola*: Past, Present and Future**
Robin Bedding CSIRO, Australia

14.10 **7** **Mechanisms responsible for *Sirex noctilio* nematode biocontrol program disruption in Australia**
Fazila Yosuf[1], **Angus Carnegie**[2]**,** Robin Bedding[3], Dick Bashford[4], Catherine Clarke[1], Geoff Gurr[1]
[1]Charles Sturt University, NSW; [2]NSW Department of Primary Industries – Forestry, Sydney, NSW; [3]CSIRO, Canberra, ACT; NSW Department of Primary Industries – Forestry, Sydney, NSW; Hobart, Tasmania.

14.30 **8** **Predicting Sirex biocontrol success in subtropical Australia: can *Deladenus siricidicola* take the heat?**
Helen Nahrung[1], Michael Ramsden[2], Manon Griffiths[3] [1]Forest Industries Research Centre, University of the Sunshine Coast, Queensland; [2]HQPlantations Pty Ltd, Queensland; [3]Horticulture & Forestry Sciences, Queensland Department of Agriculture and Fisheries, Queensland, Australia

14.50 **9-STU** **Genetic diversity in global collection of *Deladenus siricidicola***
Katrin N.E. Fitza[1], Firehiwot Eshetu[1], Jeff R. Garnas[2], Rodrigo Ahumada[3], Matthew P. Ayres[4], Flora E. Krivak-Tetley[4], Maria J. Lombardero[5], Irene Barnes[1], Helen Nahrung[6], Michael Wingfield[1] and Bernard Slippers[1] [1] Department of Biochemistry, Genetics and Microbiology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa; [2] Department of Zoology and Entomology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa; [3] Bioforest S.A., 70-C, Concepción, Chile; [4]Biological Sciences, Dartmouth College, Hanover, NH 03755, USA; [5]Departamento de Producción Vegetal, Universidad de Santiago, 27002 Lugo, Spain; [6]Forest Industries Research Centre, and Faculty of Science, Health, Education and Engineering, University of Sunshine Coast, Maroochydore, QLD 4558, Australia

15.10 **10 Potential for non-target effects using biological control nematode against *Sirex noctilio* in North America**
Ann E. Hajek[1], E. Erin Morris[1, 2], Tonya D. Bittner,[1] [1]Department of Entomology, Cornell University, Ithaca, New York, USA, [2]Department of Biological Sciences, University of New Hampshire, Durham, New Hampshire, USA

Contributed papers — Maui 3
Monday 13.30-15.30
FUNGI 1
Moderator: Jarrod Leland

13.30 **11 Global Trend in R&D of Biopesticides: 3Rs and e-Biopesticide**
Jae Su Kim[1], Se Jin Lee[1], Jong Cheol Kim1, Sihyeon Kim[1], Mi Rong Lee1, So Eun Park[1], Dongwei Li[1], Tae Young Shin[1], Taek Su Shin[2], Tae Hoon Kim[2], Pan Jung Ha[2], Tae Hyun Park[2] [1]Department of Agricultural Biology, College of Agricultural & Life Sciences, Chonbuk National University, Jeonju 561-756, Korea; [2]Crop Protection R&D Center, FarmHannong (LG Chemical Affiliated Co.), Nonsan 39-23, Korea

13.45 **12 Cancelled**

14.00 **13 Mosquito entomopathogenic fungi molecular interactions that define the outcome of infection.**
JL. Ramirez, E.J. Muturi, C. Dunlap and A. Rooney NCAUR, Agriculture Research Service, United States Department of Agriculture

14.15 **14 Effect of entomopathogenic fungi and different immunosupressors on Colorado potato beetle defense systems and ontogeny**
Olga Yaroslavtseva[1], **Vadim Kryukov**[1], Oksana Tomilova[1], Olga Polenogova[1], Maksim Tyurin[1], Maria Ganina[2], Elena Chernyak[2], Olga Luzina[2], Nariman Salakhutdinov[2], Sergey Morozov[2], Viktor Glupov[11]Institute of Systematics and Ecology of Animals, SBRAS, Novosibirsk, Russia; [2]N.N. Vorozhtsov Novosibirsk Institute of Organic Chemistry, SBRAS, Novosibirsk, Russia

14.30 **15 Arf and Rab GTPases play important roles in conidiation, trap formation, stress resistance and virulence in the nematode-trapping fungus *Arthrobotrys oligospora***
Xuewei Yang[1,2], Ni Ma[1,2], Le Yang[1,2], Ke-Qin Zhang[1,2], **Jinkui Yang**[1,2,*1]State Key Laboratory for Conservation and Utilization of Bio-Resources in Yunnan, Yunnan University, Kunming 650091, P. R. China; [2]Key Laboratory for Microbial Resources of the Ministry of Education, Yunnan University, Kunming, 650091, China

14.45 **16-STU Biological solution for entomopathogenic fungi-mediated management of Japanese pine sawyer beetle, *Monochamus alternatus***
Jong Cheol Kim, Se Jin Lee, Tae Young Shin, Sehyeon Baek, Mi Rong Lee, Sihyeon Kim, Dongwei Li, So Eun Park, and Jae Su Kim Department of Agricultural Biology, College of Agriculture & Life Sciences, Chonbuk National University, Korea

15.00 **17-STU Entomopathogenic fungal library to control longhorned tick, *Haemaphysalis longicornis***
Mi Rong Lee, Se Jin Lee, Sihyeon Kim, **Jong Cheol Kim,** So Eun Park, Dongwei Li, Sehyeon Baek, Tae Young Shin, Jae Su Kim Department of Agricultural Biology, College of Agriculture & Life Sciences, Chonbuk National University, Korea

15.15 **18-STU Targeting adult click beetles with the entomopathogenic fungus *Metarhizium brunneum*: Is it effective and are there reproductive trade-offs?**
Kari Zurowski[1], Jenny Cory[1], Todd Kabaluk[2], Alida Janmaat[31]Simon Fraser University, Burnaby, British Columbia, Canada, [2]Agriculture and Agri-Food Canada, Agassiz, British Columbia, Canada, [3] University of the Fraser Valley, Abbotsford, British Columbia, Canada

Contributed papers — Maui 1 & 2
Monday 13.30-15.30
VIRUS 1
Moderator: Zhihong Hu and Robert Harrison

13.30 **19 The development of genetically modified baculoviruses for improved control of the false codling moth, *Thaumatotibia leucotreta* in South Africa.**
Michael D. Jukes[1], Caroline M. Knox[1], Martin P. Hill[2], Sean D. Moore[2, 3], Lukasz Rabalski[4] & Boguslaw Szewczyk[4] [1]Department of Biochemistry and Microbiology, P.O. Box 94, Rhodes University, Grahamstown, 6140 South Africa [2]Department of Zoology and Entomology, Rhodes University, P.O. Box 94, Grahamstown, 6140 South Africa [3]Citrus Research International, P.O. Box 20285, Humewood, Port Elizabeth, 6013 South Africa [4]Department of molecular virology, University of Gdansk and Medical University of Gdansk, Gdańsk, Poland

13.45 **20 Establishment of Baculovirus-expressed VLPs-Induced Syncytial Formation Assay for Flavivirus Antiviral Screening**
Shiyu Dai[1],Yanfang Zhang[1], Tao Zhang[1], Zhihong Hu[1], Hualin Wang[1]*, **Fei Deng**[1]* [1]State Key laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, 430071, P.R.China.

14.00 **21-STU** **The baculovirus *per os* infectivity factor (PIF) complex and its conservation in other invertebrate large DNA viruses**
Xi Wang[1,2], Yu Shang[1], Cheng Chen[1,2], Shurui Liu[1,2], Meng Chang[1,2], Fenghua Zhang[1], Nan Zhang[1,2], Zhe Lin[3], Just M. Vlak[4], Fei Deng[1], Hualin Wang[1], Zhen Zou[3], Manli Wang[1], Zhihong Hu[1] [1]State Key Laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, China; [2]University of Chinese Academy of Sciences, Beijing 100049, China; [3]State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China; [4]Laboratory of Virology, Wageningen University and Research, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands

14.15 **22-STU** **Baculovirus oral infectivity is mediated by a complex interplay between *per os* infectivity factors**.
Bob Boogaard, Fabiola D. Ortega Murillo, Alexander L. Sminia, Jan W.M. van Lent, Monique M. van Oers Laboratory of Virology, Wageningen University and Research, the Netherlands

14.30 **23 STU** **Aedes anphevirus (AeAV): an insect-specific virus distributed worldwide in *Aedes aegypti* mosquitoes has complex interplays with *Wolbachia* and dengue virus infection in cells**
Rhys Parry, Sassan Asgari Australian Infectious Disease Research Centre, School of Biological Sciences, The University of Queensland, Brisbane, QLD 4072, Australia

14.45 **24** **Natural baculovirus coinfection in *Spodoptera ornithogalli* larvae; advances in the characterization of *Spodoptera ornithogalli nucleopolyhedrovirus* (SporMNPV) and *granulovirus* (SporGV)**
Gloria Barrera[1], Gustavo A. Araque[1], Mariano N. Belaich[2], Pablo D. Ghiringhelli,[2] [1]Corporación Colombiana de Investigación Agropecuaria - AGROSAVIA, Centro de investigación Tibaitatá – Km 14 vía Mosquera - Bogotá, Colombia. [2]Laboratorio de Ingeniería Genética y Biología Celular y Molecular-Área Virosis de Insectos, Universidad Nacional de Quilmes, Provincia de Buenos Aires, Argentina

15.00 **25** **Baculovirus as an efficient vector for gene delivery into mosquitoes**
Yu-Chan Chao[1], Nenavath Gopal Naik[1], Yu-Wen Lo[1], Tzong-Yuan Wu[2], Chang-Chi Lin[3], Szu-Cheng Kuo[3] [1]Institute of Molecular Biology, Academia Sinica, No. 128, Sec. 2, Academia Road, Nankang, Taipei 115, Taiwan, ROC; [2]Department of Bioscience Technology, Chung Yuan Christian University, Chungli 320, Taiwan, ROC; [3]Department and Graduate Institute of Microbiology and Immunology, National Defense Medical Center, Taipei 114, Taiwan, ROC

15.15 **26** **The 38K-mediated specific dephosphorylation of theviral core protein P6.9 plays an important role in the nucleocapsid assembly of autographa californica multiple nucleopolyhedrovirus**
Qingying Lai, Wenbi Wu, Ao Li, **Wei Wang**, Meijin Yuan, Kai YangState Key Laboratory of Biocontrol, Sun Yat-sen University, Guangzhou 510275, China

15.30-16.00 AFTERNOON TEA

Microbial Control Division Symposium
Monday 16.00-18.00 Pipeline

The challenge of CRB-G to palm production in the Pacific and prospects for microbial control

Organisers/Moderators: Sean Marshall & Trevor Jackson

16.00 **27** **Progress with control of a virus resistant coconut rhinoceros beetle**
Sean D.G. Marshall[1], Aubrey Moore[2], Mark Ero[3], Crispus Fanai[4], Maclean Vaqalo[5], Trevor A. Jackson[1] [1]Forage Science, AgResearch, Lincoln Research Centre, Christchurch, New Zealand; [2] College of Natural and Applied Sciences, University of Guam, Guam, USA; [3]Papua New Guinea Oil Palm Research Association, Kimbe, Papua New Guinea; [4]Biosecurity Solomon Islands, Ministry of Agriculture and Livestock, Honiara, Solomon Islands; [5]Land Resources Division, Pacific Community, Suva, Fji

16.15 **28** **Attempted microbial control of coconut rhinoceros beetle, *Oryctes rhinoceros*, Biotype G on Guam using *Oryctes rhinoceros* nudivirus and *Metarhizium majus***
Aubrey Moore[1], Sean D. G. Marshall[2], Roland Quitugua[1], Ian Iriarte,[1] [1]College of Natural and Applied Sciences, University of Guam, Mangilao, Guam, USA;[2] Forage Science, AgResearch, Lincoln Research Center, Christchurch, New Zealand

16.30 **29** **Biotype and diversity of *Oryctes rhinoceros* in Japan**
Madoka Nakai, Institute of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan

16.45 **30** **CRB damage and resistance assessment in the Palau Archipelago**
Christopher Kitalong[1], Justin Omak Ramarui[1], Jason Ngiramengior[1], Balang Skey[1], Nelson Masang[1,2], Shizu Watanabe[2], Michael Melzer[2], **Madoka Nakai**[3], Joel Miles[1]. [1]Pacific Academic Institute for Research/Palau Community College STEP-UP Lab. Koror, PALAU. [2]Department of Plant and Environmental Protection Sciences,

University of Hawaii, Manoa, USA. [3]Depart of Insect Pathology, Tokyo University of Agriculture Technology, JAPAN

17.00 **31** **Infectivity of Malaysian *Oryctes* nudivirus (OrNV) propagated in insect cell line DSIR-HA-1179 against the rhinoceros beetle, *Oryctes rhinoceros***
Nur Ain Farhah Ros Saidon Khudri[1], Norman Kamarudin[1], Sean Marshall[2] and Ramle Moslim[3]. [1] Biological Research Division, Malaysian Palm Oil Board, 6, Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang Selangor. [2] Innovative Farm Systems, AgResearch Ltd, Lincoln Research Centre, Private Bag 4749, Christchurch 8140, New Zealand. [3] Integration Research and Extension Division, Malaysian Palm Oil Board, 6, Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang, Selangor

17.15 **32** **Coconut Rhinoceros Beetle (CRB) control efforts in oil palm: Papua New Guinea (CRB-P) versus Solomon Islands (CRB-G)**
Mark Ero and Luc Bonneau Papua New Guinea Oil Palm Research Association, Kimbe, Papua New Guinea

17.30 **33** **The status of Coconut Rhinoceros Beetle, *Oryctes rhinoceros* (L) Scarabaeidae : Dynastinae, in Solomon Islands**.
Francis Tsatsia1, Helen Tsatsia2, Hilda Wratten2, Bob Macfarlane3 1.Biosecurity Solomon Islands, MAL, Honiara 2.Agriculture Research, MAL, Honiara.3.Retired, Coordinator of the CRB Response

17.45 **34** **Challenge of a new biotype of the coconut rhinoceros beetle (CRB-G) to the Pacific**
Maclean Vaqalo[1], Visoni Timote[1], Fereti Atu[1], Sean Marshall[2], Trevor Jackson[2] [1]Land Resource Division, Pacific Community, Suva, Fiji; [2]Forage Science, AgResearch, Lincoln Research Centre, Christchurch, New Zealand

Contributed papers — Maui 1 & 2
Monday 16.00-18.00

BENEFICIAL INVERTEBRATES & MICROSPORIDIA 1

Moderator**:** Helen Hesketh and Lyric Bartholomay

16.00 **35-STU** **A possible new species of *Tubulinosema* (Microsporidia: Tubulinosematidae) affecting silkworms (*Bombyx mori*) in Brazi**l
Maximiano C. Cassal[1], Lidia M. Fiuza[2], Kazuhiro Iiyama[1] and Chisa Yasunaga-Aoki,[1 1]Laboratory of Insect Pathology and Microbial Control, Institute of Biological Control, Faculty of Agriculture, Kyushu University, Fukuoka, Japan [2]Rio-Grandense Rice Institute (IRGA), Cachoeirinha, RS, Brazi

16.15 **36-STU** **Transcriptomic analysis of *Rozella allomycis* and spliceosomal diversity of early-diverging fungi**
Whelan, T.[1], Quandt, C. A.[2], James, T. Y.[2] and **Fast, N. M,**.[11] Biodiversity Research Centre and Department of Botany, University of British Columbia, Canada [2] Department of Ecology and Evolutionary Biology, University of Michigan

16.30 **37** **Controlling the pandemic of the microsporidian *Enterocytozoon hepatopenaei* in shrimp aquaculture: from molecular understanding to practical solutions**
Ornchuma Itsathitphaisarn[1, 2], Pattana Jaroenlak[1, 2], Natthinee Munkongwongsiri[2, 3], Piyachat Sanguanrut[2, 3], Anuphap Prachumwat[2, 3], Bryony A. P. Williams[4], Grant D. Stentiford[5], Timothy W. Flegel[2,6], Kallaya ritunyalucksana[3,6] [1]Department of Biochemistry, Faculty of Science, Mahidol University, Bangkok, Thailand [2] Center of Excellence for Shrimp Molecular Biology and Biotechnology (Centex Shrimp), Faculty of Science, Mahidol University, Bangkok, Thailand [3] Shrimp–Pathogen Interaction Laboratory (SPI), National Center for Genetic Engineering and Biotechnology (BIOTEC), Bangkok, Thailand [4] Biosciences, College of Life and Environmental Sciences, University of Exeter, Exeter, UK [5] Center for Environment, Fisheries and Aquaculture Science (Cefas), Weymouth, Dorset, UK [6]National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Pathumthani, Thailand

16.45 **38** **The influence of microsporidian pathogens from commercially available lady beetles on non-target insect predators**
Susan Bjornson Department of Biology, Saint Mary's University, 923 Robie Street, Halifax, Nova Scotia, Canada

17.00 **39** **A new species of microsporidia from the mosquito *Uranotaenia lowii* is related to the *Hazardia* clade**
James J. Becnel and Neil D. Sanscrainte Center for Medical, Agricultural and Veterinary Entomology, US Department of Agriculture, Agricultural Research Service, Gainesville, Florida 32608

17.15 **40** **Diversity of pathogens associated with edible long-horned grasshoppers in East Africa**
Alfonce Leonard[1, 2], Fathiya *M.* Khamis[1], Samuel Kyamanywa[2], Sunday Ekesi[1], Komi K. M. Fiaboe[1], Chrysantus Tanga Mbi[1], James P. Egonyu[2], **Sevgan Subramanian**[1* 1] International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya [2]Makerere University, Kampala, Uganda

18.00-20.00 Southbreak
ICTV Baculoviridae/
Nudiviridae Study Group meeting

20.00-22.00 Maui 3
Viruses Division business meeting

20.00-22.00 Northbreak
Microsporida Division business meeting

20.00-22.00 Cloudbreak
Bacteria Division business meeting

20.00-22.00 Maui 2
Microbial Control Division business meeting

Tuesday 14 August 2018

Virus Division Symposium Pipeline
Tuesday 08.00-10.00

Interactions between arboviruses and their vectors

Organisers/Moderators: Karyn Johnson & Rollie Chem

8.00 **41** **Barriers to arbovirus infection in mosquitoes**
Rollie J. Clem Division of Biology, Kansas State University, Manhattan, Kansas USA

8.30 **42** **Mosquito and viral determinants that condition host specificity, tissue tropisms and transmission: spotlight on the flaviviruses**
Lyric Bartholomay[1], Stephen Peinado[1], Paul Airs[1], Bradley Blitvich[2] [1]School of Veterinary Medicine, University of Wisconsin-Madison, Madison Wisconsin, USA; [2]College of Veterinary Medicine, Iowa State University, Ames Iowa, USA

9.00 **43** **Commensal Viruses of Mosquitoes: Host Restriction, Transmission, and Interaction with Arboviral Pathogens**
Jody Hobson-Peters[1], Sonja Hall-Mendelin[2], Andrew F van den Hurk[2], Helle Bielefeldt-Ohmann[1], Breeanna J McLean[1], Agathe M G Colmant[1], Cameron E Webb[3], Caitlin A O'Brien[1], Jessica J Harrison[1], David Warrilow[2], Thisun B H Piyasena[1], Alexander A Khromykh[1] and Roy A Hall[1] [1]Australian Infectious Diseases Research Centre, School of Chemistry and Molecular Biosciences, The University of Queensland, St Lucia, QLD, Australia; [2]Public Health Virology Laboratory, Forensic and Scientific Services, Department of Health; [3]Department of Medical Entomology, The University of Sydney and Westmead Hospital

9.30 **44** **Contribution of microRNAs in mosquito-virus interactions**
Sassan Asgari[11]Australian Infectious Disease Research Centre, School of Biological Sciences, The University of Queensland, Brisbane QLD 4072

Contributed papers Maui 1 & 2
Tuesday 8.00-10.00

BACTERIA 1

Moderator: Juan Ferre

8.00 **45** **Discovery of New Insecticidal Traits from Bacteria**
Vadim Beilinson and AgBiome team AgBiome Inc., 108 T.W. Alexander Dr. Bldg 1, RTP, NC, USA

8.15 **46** **Do the differences between naturally occurring and GM Cry insecticidal toxins impact to specificity?**
Jonathan R. Latham [1], Madeleine Love[2], Angelika Hilbeck[3] [1]The Bioscience Resource Project, Ithaca, NY, 14850, USA [2]Independent scholar; Melbourne, Victoria, Australia; [3]Federal Institute of Technology (ETH) Zurich, Switzerland.

8.30 **47-STU** **Identification of genes involved in the global secretion of entomopathogenic virulence factors in *Yersinia entomophaga***
Marion Schoof[1, 3], *Campbell Sheen[2], Maureen O'Callaghan[3], Travis Glare[1], and Mark Hurst* [3 1] Bio-Protection Research Centre, PO Box 85084, Lincoln University, Lincoln 7647, Christchurch, New Zealand, [2] Protein Science and Engineering, Callaghan Innovation, Christchurch, New Zealand, [3] AgResearch, Farm Systems & Environment, Lincoln Research Centre, Christchurch 8140

8.45 **48** **Non-venomous insect sPLA$_2$ and its physiological functions in development and immunity**
Yonggyun Kim Department of Plant Medicals, Andong National University, Andong 36729, Korea

9.00 **49** **Novel mosquitocidal toxins from Clostridia**
Estefania Contreras-Navarro, Jianwu Chen and **Sarjeet Gill** , University of California, USA.

9.15 **50** **Isolation of cell wall encapsulated or purified Cry5B crystals from asporogenous *Bacilli* for use as a anthelminthic drug**
Ambily Abraham, Deysy Tatiana Pinto Rodriguez, Yan Hu, Hanchen Li, Kelly Flanagan, David Gazzola, Tasia Kellogg, Gary Ostroff and Raffi Aroian, Program of Molecular Medicine, University of Massachusetts Medical School, Worcester, MA, 01605, USA

9.30 **51** **Using an inactivated soil bacterium to kill parasitic nematodes**
Yan Hu[1], David Gazzola[1], Hanchen Li[1], Tasia Kellogg[1], Kelly Flanagan[1], Ambily Abraham[1], Martin K. Nielsen[2], Anne Zajac[3], Joseph F. Urban[4], Katherine Petersson[5], Gary Ostroff[1], and Raffi Aroian*[1] [1]Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, MA, USA, [2]M.H. Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky, Lexington, KY, USA, [3]Dept. Biomedical Sciences and Pathobiology, VA-MD College of Veterinary Medicine, Virginia Tech, Blacksburg, VA, USA, [4]USDA, Agricultural Research Service, Beltsville Human Nutrition Research Center, Diet, Genomics, and Immunology Laboratory, Beltsville, MD, USA, [5]Dept. Fisheries, Animal & Veterinary Science, University of Rhode Island, 120 Flagg Road, 177 CBLS, Kingston, RI, USA,

9.45 **52** **Investigation of the role of known insect receptors in mediating the toxicity of nematicidal pore-forming toxins**
Anand Sitaram, You-Mie Kim, Raffi V. Aroian Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, Massachusetts, USA

10-10.30 MORNING TEA

Bacterial Division Symposium Pipeline
Tuesday 10.30-12.30

Insect resistance mechanisms to Bt.

Organisers/Moderators: David Heckel and Simon Baxter

10.30 **53** **Combining deleterious ABC transporter C2 alleles of independent origin causes field resistance to insecticidal Bt toxins**
Yang Dong[1], Christopher Ward[1], Zhaojiang Guo[2], Mark Blaxter[3], Neil Crickmore[4], Chris Jiggins[5], Ron Mau[6], Ben Raymond[7], Tony Shelton[8], Robin Shimabuku[6], Youjun Zhang[2], David G. Heckel[9], and **Simon W. Baxter[1]**[1]School of Biological Sciences, University of Adelaide, Australia; [2]Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences (CAAS), Beijing; [3]GenePool, University of Edinburgh, UK; [4]School of Life Sciences, University of Sussex, UK; [5]Department of Zoology, University of Cambridge, UK; [6]College of Tropical Agriculture and Human Resources, University of Hawaii, USA; [7] University of Exeter, Penryn Campus, UK; [8]College of Agriculture and Life Sciences, Cornell University, USA; [9]Department of Entomology, Max Planck Institute for Chemical Ecology, Jena, Germany

10.50 **54** **Bt Resistance in Australian Insect Pests**
Sharon J Downes[1], Tom Walsh[2], Wee Tek Tay[3], Amanda Padovan[2] [1]CSIRO Agriculture and Food, Narrabri, Australia; [2]CSIRO Land and Water, Canberra, Australia; [3]CSIRO Health and Biosecurity, Canberra, Australia

11.10 **55** **MAPK signaling pathway *trans*-regulates differential expression of aminopeptidases N and confers resistance to *Bacillus thuringiensis* Cry1Ac toxin in diamondback moth**
Zhaojiang Guo, Shi Kang, Dan Sun, Youjun Zhang. Department of Plant Protection, Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing 100081, China

11.30 **56** **Field-evolved resistance to Bt corn in fall armyworm: mechanism, dispersal and biological implications**
Rahul Banerjee[1], Heba Abdelgaffar[1], Omaththage Perera[2], Lucas Hietala[1], Caroline Placidi de Bortoli[1], **Juan Luis Jurat-Fuentes[1]** [1]Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, Tennessee, USA [2]Southern Insect Management Research Unit, USDA-ARS, Stoneville, MS, USA

11.50 **57** **Mutations of ABC transporters and Bt resistance in cabbage loopers**
Ping Wang[1], Xiaoli Ma[1], Xiaowei Yang[1], Wenbo Chen[2], Wendy Kain[1], Xiaozhao Song[1], Hannah Chu[1] and Zhangjun[2] Fei[1]Department of Entomology, Cornell University, Geneva, NY 14456, USA; [2]Boyce Thompson Institute, Ithaca, NY 14853, USA

12.10 **58** **Function and role of ATP-binding cassette transporters as a Cry toxins receptor**
Haruka Endo[1], Shiho Tanaka[1], Satomi Adegawa[1], Xiaoyi Li[1], Kenji Watanabe[2] and **Ryoichi Sato**[1] [1]Graduate School of Bio-Applications and Systems Engineering, Tokyo University of Agriculture and Technology, Tokyo, Japan; [2]Institute of Agrobiological Sciences, National Agriculture and Food Research Organization, Ibaraki, Japan.

Contributed papers Maui 1 & 2
Tuesday 10.30-12.30

VIRUS 2

Moderator**:** Martin Erlandson and Monique van Oers

10.30 **59** **Baculoviruses as quasispecies: dynamics and selection *in vivo*.**
Caroline Hauxwell, Chris Noune, Boyd Tarlinton, James McGree Queensland University of Technology (QUT), Brisbane 4000, Queensland, Australia

10.45 **60-STU** **Inhibition of adenosine pathway increases host susceptibility to baculovirus infection**
Chia-Chi Tai, Yueh-Lung Wu Department & Graduate Institute of Entomology, National Taiwan University, Republic of China (Taiwan)

11.00 **61-STU** **Clathrate cage-like apparatus of baculovirus IE2 as a versatile cross-phylum gene transactivation system**
Chih-Hsuan Tsai, Sung-Chan Wei, Yu-Chan ChaoInstitute of Molecular Biology, Academia Sinica, Taipei 115, Taiwan, ROC.

11.15 **62** **Baculovirus PTP2 functions as a pro-apoptotic protein**
Yue Han[1], Vera I.D. Ros[1], Stineke van Houte[2], **Monique M. van Oers**[1 1]Laboratory of Virology, Wageningen University & Research, Wageningen, the Netherlands [2]Centre for Ecology and Conservation, Biosciences, University of Exeter, Penryn, Cornwall, United Kingdom

11.30 **63** **The nuclear import mechanism of AcMNPV VP80**
Wei Shao[1,2], Lihong He[1,2], Just M. Vlak[3], Fei Deng[1], Hualin Wang[1], Zhihong Hu[1] and **Manli Wang**[1] [1]State Key Laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, P. R. China; [2]University of Chinese Academy of Sciences, Beijing 100049, P. R. China; [3]Laboratory of Virology, Wageningen University and Research, Droevendaalsesteeg 1, 6708 PB

11.45 **64** **Functional analysis of the lysine residues of Autographa californica nucleopolyhedrovirus (AcMNPV) viral ubiquitin**
Siddhartha Biswas[1], Leslie G. Willis[2], Martin A. Erlandson[3], **David A. Theilmann**[1,2]. [1]Plant Science, Faculty of Land Food Systems, University of British Columbia, Vancouver, BC, Canada; [2]Summerland Research and Development Centre, Agriculture and Agri-Food Canada, Box 5000, Summerland BC, Canada V0H 1Z0; [3]Saskatoon Research and Development Centre, Agriculture and Agri-Food Canada, Saskatoon, Saskatchewan, Canada S7N 0X2

12.00 **65** **Mamestra configurata nucleopolyhedrovirus -B ORF 54, characterization and comparative analysis of homologues in other baculovirus species**
Martin A. Erlandson[1], Rahul P. Hepat [2], Julianne Peralta[1], Douglas Baldwin[1], David A. Theilmann[2.] [1]Saskatoon Research and Development Centre, Agriculture and Agri-Food Canada, Saskatoon, Saskatchewan, Canada; [2] Research and Development Centre, Agriculture and Agri-Food Canada, Summerland, British Columbia, Canada

12.15 **66** **Identification and characterization of nucleolus localization signal of Autographa californica Multiple Nucleopolyhedrovirus LEF-5 protein**
Guoqing Chen, Pei Li, Qing Yan, Lijuan Wu, **Guozhong Feng** State Key Laboratory of Rice Biology, China National Rice Research Institute, Hangzhou, China.

12.30-17.00 EXCURSIONS

17.00-17.10 BUSES DEPART FROM QT

19.00-22.00 BBQ DINNER

WEDNESDAY 15 August 2018

Bacterial Division Symposium Pipeline
Wednesday 08.00-10.00

Insecticidal protein structures

Organisers/Moderators: Mark Hurst and Trevor Jackson

8.00 **67** **Cryo-EM structure of an insecticidal toxin–ion channel complex reveals thecomplex molecular basis of allosteric modulation of channel gating**
Glenn F King Institute for Molecular Bioscience, The University of Queensland, St Lucia QLD 4072, Australia

8.20 **68** **Pleurotolysin: a pore forming toxin from the carnivorous oyster mushroom**
Stephanie C. Kondos[1], Natasha Lukuyonova[2], Bradley A. Spicer[1], Susan M. Ekkel[1], Helen Saibil[2],**Michelle A. Dunstone**[1] [1]Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria 3800, Australia; [2]Birkbeck College, London, UK

8.40 **69-STU**- **Lessons from the vertebrate immune system: how the membrane attack complex shoots an evolutionary moving target**
Bradley A. Spicer[1], Ruby HP Law[1], Charles Bayly-Jones[1], Tom T. Caradox-Davies[3], Paul J. Conroy[1], Susan M Ekkel[1], Natalya Dudkina[2], James C. Whisstock[1], Helen Saibil[2], Michelle A. Dunstone[11]Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria 3800, Australia; [2]Birkbeck College, London, UK; [3]Australian Synchrotron, Clayton, Victoria 3168, Australia

9.00 **70** **Cryo-EM structures of the pore-forming ABC toxin from *Yersinia entomophaga***
Sarah J Piper,[2] Lou Brillault,[1,2] Rosalba Rothnagel,[2] Tristan I Croll,[3] Joseph K Box,[1] Sebastian Scherer,[4] Kenneth N Goldie,[4] Sandra A Jones,[5] Femke Schepers,[6] Jason N Busby,[7] Julie E

Dalziel,[8] J Shaun Lott,[7] Ben Hankamer,[2] Henning Stahlberg,[4] Mark RH Hurst,[5] **Michael J Landsberg**[1,2] [1]School of Chemistry and Molecular Biosciences, The University of Queensland, St Lucia 7 Queensland 4072, Australia. [2]Institute for Molecular Bioscience, The University of Queensland, St Lucia Queensland 4072, Australia. [3]Cambridge Institute of Medical Research, University of Cambridge, Cambridge Cambridgeshire CB2 0XY, United Kingdom 12. [4]Centre for Cellular Imaging and NanoAnalytics, Biozentrum, University of Basel, 4058 Basel, Switzerland. [5]Forage Science Group, AgResearch, Christchurch 8140, New Zealand. [6]Faculty of Science, University of Leiden, 2300 RA Leiden, The Netherlands. [7]School of Biological Sciences, University of Auckland, Auckland 1142, New Zealand. [8]Food & Bio-based Products Group, AgResearch, Palmerston North 4442, New Zealand.

9.20 **71** **Insights into the cellular recognition patterns of Yentc, an insecticidal pore-forming toxin**
Irène R. Chassagnon[1], Sarah J. Piper[1,2], Michael J. Landsberg[1,21] School of Chemistry and Molecular Biosciences, The University of Queensland,; [2]Institute for Molecular Bioscience, The University of Queensland, QLD Australia

9.40 **72** **Insecticidal ABC toxin complexes encapsulate a variety of toxins**
J. Shaun Lott[1], Sean Marshall[2] Jason N. Busby[1], Sarah Trevelyan[1] Mark R. H. Hurst[2] [1]School of Biological Sciences, The University of Auckland, Auckland, New Zealand; [2]Innovative Farming Systems, AgResearch, incoln, New Zealand.

Contributed papers — Maui 1 & 2

Wednesday 8.00-10.00

MICROBIAL CONTROL 1

Moderator: Dietrich Stephan

8.00 **73** **A novel biopesticide to control black beetle in pasture**
Sarah Mansfield[1], Philippa J. Gerard[2], Mark R.H. Hurst[1], Derrick J. Wilson[2], David A. Wright[1], Jayanthi Swaminathan[1], Michael J. Wilson[2], Chikako van Koten[1] [1]AgResearch, 1365 Springs Road, Lincoln, New Zealand; [2]AgResearch, 10 Bisley Road, Ruakura, New Zealand

8.15 **74** ***Metarhizium anisopliae*** **infection reduces Trypanosoma congolense reproduction in *Glossina fuscipes fuscipes* and its vector competence**
Wamiti L. G.[1, 2], **Khamis F. M**. [1*], Abd-alla A. M. M. [3], Ombura F. L. O. [1], Akutse K. S. [1], Subramanian S. [1], Odiwuor S. O. [2], Ochieng S. J. [1, 4], Ekesi S. [1] and Maniania N. K. [1, a]
[1]International Centre of Insect Physiology and Ecology (*icipe*), P.O. Box 30772-00100, Nairobi, Kenya [2]Mount Kenya University, P.O. Box 324 – 01000 Thika, Kenya [3]Insect Pest Control Laboratory, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, International Atomic Energy Agency, Wagramerstraße 5, A-1400 Vienna, Austria [4]Kenyatta University, Medical Physiology Department, P.O. Box 43844 – 00100, Nairobi [a]Current address: 1501 Fisher Avenue, Ottawa, ON, K2C 3M8, Canada

8.30 **75** **NoVil: The hunt for weevil control**
Nguya K. Maniania, Angela Demarse, Andrei Darie, Ishtiaq M. Rao Crop Defenders Ltd., Windsor, Ontario, Canada

8.45 **76-STU** Cancelled

9.00 **77** **The 'MycoHarvester': a device for optimising mycopesticide storage and formulation**
Roy Bateman VBS (Agriculture) Ltd., Twickenham, UK

9.15 **78-STU** **Yeast-baculovirus synergism: Investigating mixed infections for improved management of the false codling moth, *Thaumatotibia leucotreta***
Marcel van der Merwe[1], Caroline M. Knox[1], Martin P. Hill[2], Sean D. Moore[2, 3] [1]Department of Biochemistry and Microbiology, P.O. Box 94, Rhodes University, Grahamstown, 6140 South Africa [2]Department of Zoology and Entomology, Rhodes University, P.O. Box 94, Grahamstown, 6140 South Africa [3]Citrus Research International, P.O. Box 20285, Humewood, Port Elizabeth, 6013 South Africa

9.30 **79-STU** Cancelled

9.45 **80-STU** Cancelled

9.30 **Where do *Isaria fumosorosea* and *Burkholderia rinojensis* fit in with chemical and biological pesticides for zucchini pest management?**
Surendra Surendra K. Dara[1], Sumanth S. R. Dara[2], Suchitra S. Dara[2] and Edwin Lewis[3]. [1]University of California Cooperative Extension, San Luis Obispo, CA, USA; [2]Global Agricultural Solutions, Bakersfield, CA, USA; [3]University of Idaho, Moscow, ID, USA

Contributed papers Maui 3

Wednesday 8.00-10.00

VIRUS 3

Moderators: Vera Ros and Robert Possee

8.00 **81** **Use of whole genome sequences for baculovirus species demarcation and taxonomy**
Jörg T. Wennmann[1], Jens Keilwagen[2], Johannes A. Jehle[1] [1]Julius Kühn Institute, Federal Research Centre for Cultivated Plants, Institute for Biological Control, Darmstadt, Germany; [2]Julius Kühn Institute, Federal Research Centre for Cultivated Plants, Institute for Biosafety and Plant Biotechnology, Quedlinburg, Germany

8.15 **82** **Baculovirus invasion of the lepidopteran central nervous system**
Yue Han[1], Jitte Groothuis[1], Hanneke Suijkerbuijk[1], Yijing Wang[1], Jan W.M. van Lent[1], Hans M. Smid[2],**Vera I.D. Ros**[1]1Laboratory of Virology, Wageningen University & Research, Droevendaalsesteeg 1, 6708 PB Wageningen, the Netherlands; [2]Laboratory of Entomology, Wageningen University & Research, Droevendaalsesteeg 1, 6708 PB Wageningen, the Netherlands

8.30 **83-STU** ***W*hole genome analysis of a *baculovirus i*solated from a persistently infected insect cell line**
Raquel Arinto-Garcia[1*], Carina Bannach[1], Daniel Leite[1], Chris Hawes[1], Linda King[1], Robert Possee[2] [1] Dept. Biological & Medical Sciences, Oxford Brookes University, Oxford UK [2]Oxford Expression Technologies Ltd, Oxford, UK

8.45 **84-STU** **Transcriptome analysis of an insect cell line harbouring a persistent baculovirus infection**
Carina Bannach[1], Raquel Arinto Garcia[1], Kan Bao[2], Zhangjun Fei[2], Gary W. Blissard[2], Linda A. King[1] and Robert D. Possee[1,3] [1] Department of Biological and Medical Sciences, Oxford Brookes University, Oxford, United Kingdom, [2] Boyce Thompson Institute at Cornell University, Ithaca, New York, United States of America, [3] Oxford Expression Technologies Ltd., Oxford, United Kingdom

9.00 **85-STU** **Integration of transcriptomics and metabolomics reveals a role of cellular methylation process during *Bombyx mori nucleopolyhedrovirus* infection**
Hiroyuki Hikida[1], Yutaka Suzuki[2], Munetaka Kawamoto[1], Toru Shimada[1], and Susumu Katsuma[1] [1]Department of Agricultural and Environmental Biology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113-8657, Japan; [2]Department of Computational Biology and Medical Sciences, Graduate School of Frontier Sciences, The University of Tokyo, 5-1-5 Kashiwanoha, Kashiwa, Chiba 277-0882, Japan

9.15 **86** **Structural Proteins of the Aedes sollicitans Nucleopolyhedrovirus (AesoNPV)**
Omaththage Perera[1], James J. Becnel[2], Neil Sanscrainte[2], and Alden Estep[3] [1] Southern Insect Management Research Unit, USDA-ARS, 141 Experiment Station Road, PO Box 346, Stoneville, MS 38776. *op.perera@ars.usda.gov* [2]Center for Medical, Agricultural and Veterinary Entomology, USDA/ARS, 1600-1700 SW 23rd Drive, Gainesville, FL 32608 [3]Navy Entomology Center of Excellence, CMAVE Detachment, 1600-1700 SW 23rd Drive, Gainesville FL

9.30 **87** **The Se301 cell Aggregation induced by *Spodoptera exigua* multiple *Nucleopolyhedrovirus***
Zhihong Huang, Mengjia Pan, Wenbi Wu, Meijin Yuan, Kai Yang State Key Laboratory of Biocontrol, Sun Yat-sen University, Guangzhou 510275, China

9.45 **88** **Uncleaved GP64 signal peptide of *Bombyx mori* nucleopolyhedrovirus is required for cell membrane localization and its special dependence to *cholesterol* recognition amino acid consensus**
Jinshan Huang, Lin Liu, Wenbin Nan, Xudong Tang, Xingjia Shen, Bifang Hao, Sericultural research Institute, Jiangsu University of Science and Technology, Zhenjiang, Jiangsu, PRC

10-10.30 MORNING TEA

Beneficial Insects Division Symposium Pipeline

Wednesday 10.30-12.30

Health issues of bee and non-bee pollinators

Organisers/Moderators: Helen Hesketh & Elke Genersch

10.30 **89** **Viral landscape of Varroa-free Australian honey bees**
John M. K. Roberts[1], Denis L. Anderson[2], Peter A. Durr[3] [1]Commonwealth Scientific and Industrial Research Organisation, Canberra, ACT, 2601, Australia; [2]ADFCA, Research and Development Division, Al Ain, UAE; [3]CSIRO, Australian Animal Health Laboratory, Geelong, VIC, 3219, Australia

11.00 **90** **The role of Deformed wing virus (DWV) in Varroa tolerant honey bee populations and its spread beyond bees into the wider insect community.**
Laura E. Brettell[1,2], Jessika Santamaria[3], Ethel Villalobos[3], Gideon Mordecai[4,5]; Declan

Schroeder[4,6]; Stephen J Martin[2] [1]Hawkesbury Institute for the Environment, Western Sydney University, Richmond, NSW, Australia; [2]School of Environment and Life Sciences, University of Salford, Manchester, UK; [3]University of Hawaii at Manoa, Honolulu, HI, USA; [4] The Marine Biological Association of the UK, Plymouth, UK; [5]Earth Ocean and Atmospheric Sciences, University of British Colombia, Vancouver, Canada; [6]Veterinary Population Medicine Department, University of Minnesota, MN, USA

11.35 **91 A natural product inhibited the replication and expression of Israeli acute paralysis virus**
Yang Sa [1,2], Xu Xiang[1,2], Zhao Hongxia [1,2,3], Deng Shuai [1,2], Chu Yanna [1,2], Yang Dahe [1,2,4], Wang Xinling [1,2], Zhao Di [1,2], Diao Qingyun [1,2], **Hou Chunsheng** [1,2*1]Institute of Apicultural Research, Chinese Academy of Agricultural Sciences, Beijing 100093, P. R. China; [2]Key Laboratory of Pollinating Insect Biology, Ministry of Agriculture, Beijing, 100093, P. R. China; [3] Guangdong Institute of Applied Biological Resources, Guangzhou, 510260,P. R. China; [4] Graduate School of Chinese Academy of Agricultural Sciences, Beijing, 100081, P. R. China

11.50 **92 Enhancement of chronic bee paralysis virus levels in honeybees acute exposed to imidacloprid: a chinese case study**
Diao Qingyun [1,2#], Li Beibei [1,2#], Zhao Hongxia [3], Wu Yanyan [1,2], Guo Rui [4], Dai Pingli [1,2], Chen Dafu [4], Wang Qiang [1,2,] Hou Chunsheng [1,2*] [1]Institute of Apicultural Research, Chinese Academy of Agricultural Sciences, Beijing 100093, P. R. China; [2] Key Laboratory of Pollinating Insect Biology, Ministry of Agriculture, Beijing, 100093, P. R. China; [3] Guangdong Key Laboratory of Animal Conservation and Resource Utilization, Guangdong Public Laboratory of Wild Animal Conservation and Utilization, Guangdong Institute of Applied Biological Resources, Guangzhou, 510260, P.R. China; [4] College of Bee Science, Fujian Agricultural and Forestry University, Fuzhou, 350002 ,P. R. China

12.05 **93 Evidence of deformed wing virus (DWV) – free honey bee populations in the Pacific region**
John M. K. Roberts Commonwealth Scientific and Industrial Research Organisation, Canberra, ACT, 2601, Australia;

Contributed papers Maui 1 & 2
Wednesday 10.30-12.30

MICROBIAL CONTROL 2

Moderator: Dietrich Stephan

10.30 **94 BASF's bio-insecticide portfolio – Nemasys and Velifer – new products for the Australian market**
Sarah Anderson[,1], Diana K Londono[2], Shaun D Berry[2] [1]BASF Australia Ltd., 1205 Old Pacific Highway, 2250 Somersby, Australia; [2]BASF Corporation, 26 Davis Drive, Durham, North Carolina, USA

10.45 **95-STU Influence of host nutrition on mixed pathogen interactions: disease outcome and pathogen replication**
Pauline Deschodt*,* Olivia Walker, Alana Breitkreutz, Jessi Ly and Jenny Cory Department of Biological Sciences, Simon Fraser University, Burnaby, Canada

11.00 **96 CRISPR/Cas-mediated Gene Editing of ATP Binding Cassette Transporter type-A3 in *Spodoptera frugiperda* results in high resistance to Cry2Ab-like protein.**
John Mathis, Deirdre Kapka-Kitzman, Cathi Clark, Jean Dyer, Joe Zhao, Amit Sethi and Mark Nelson Corteva Agriscience™, Agriculture Division of DowDuPont, Johnston, Iowa USA

11.15 **97 Investigation into specificity determinants of Vip3Bc1**
Marc Zack[1], Megan Sopko[1], Scott Bevan[1], Ted Letherer[1], Sek Yee Tan[1], Yolanda Bel[2], Baltasar Escriche[2], and Ken Narva[1] [1]Corteva Agriscience Agriculture Division of DowDuPont, Indianapolis, Indiana, USA: ; [2] ERI BioTecMed, Dep.Genética, Universitat de València. C/ Dr. Moliner, 50, 46100-Burjassot, Valencia Spain

11.30 **98 Selection for UV-resistance in the Cryptophlebia leucotreta betabaculovirus for a more persistent biopesticide**
Patrick Mwanza[1], Gill Dealtry[1], Michael Lee[2] and **Sean Moore**[3, 4] [1]Department of Biochemistry and Microbiology, Nelson Mandela University, [2]Centre for High Resolution Transmission Electron Microscopy, Nelson Mandela University, Port Elizabeth South Africa; [3] Citrus Research International, Port Elizabeth, South Africa; [4]Centre for Biological Control, Department of Zoology and Entomology, Rhodes University, South Africa.

11.45 **99 The potential use of a novel alphabaculovirus as a microbial control agent against three economically important tortricid pests**
T.Marsberg[1,3], M.D. Jukes[2], M.P. Hill[1], C. Knox[2], S.D. Moore[1,3], B. Szewczyk[4], L. Rabalski[4] and J. A. Jehle[5] [1]Centre for Biological Control, Department of Zoology and Entomology, Rhodes University, P.O. Box 94, Grahamstown, 6140, South Africa. [2]Department of Biochemistry and Microbiology, Rhodes University, P.O. Box 94, Grahamstown, 6140 South Africa. [3]Citrus Research International, P.O. Box 20285, Humewood, Port Elizabeth, 6013 South Africa. [4]Department of Molecular Virology, Intercollegiate Faculty of Biotechnology and Medical University of Gdansk, Kladki 24, Poland. [5]Institute for Biological Control, Federal Research Centre for

Cultivated Plants, Julius Kühn-Institut, 64287, Darmstadt, Germany

12.00 **100 Developing RNA interference as a species-specific biological insecticide**
Ernesto Soto[1], James Baum[2], Jodi Beattie[2], Stephen Beishir[2], Michelle Gasper[2], Steven Halls[2], Jennifer Howard[2], Joanna Pawlak[2], Tanusri Samanta[2], **Gary Ostroff**[1] [1] Program in Molecular Medicine, UMass Medical School, [2] Monsanto Corporation

12.15 **101 Formation and dispersion mechanisms of *Bacillus thuringiensis* biofilm and its potential biocontrol application**
Tianpei Huang, Xiong Guan State Key Laboratory of Ecological Pest Control for Fujian and Taiwan Crops & Key Laboratory of Biopesticide and Chemical Biology of Ministry of Education, College of Life Sciences & College of Plant Protection, Fujian Agriculture and Forestry University, Fuzhou 350002, China

12.30-13.30 LUNCH (NOT supplied)

12.30-13.30 Maui 3

Nematode Division business meeting

POSTERS

WEDNESDAY 16 AUGUST 13.30-15.30
CONFERENCE FOYER

BACTERIA

BA-1 A toxin-antitoxin system is essential for the stability of mosquitocidal plasmid pBsph of *Lysinibacillus sphaericus*

Pan Fu , Yong Ge , Yimin Hu, Zhiming Yuan, **Xiaomin Hu** Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430070, China

BA-2 Binding studies, insect bioassays, and field trials on a modified Vip3C protein and other proteins active against *Spodoptera frugiperda* and *Helicoverpa armigera*

Theodore W. Kahn[1,†], Maissa Chakroun[2,†,i], Jayme Williams[1], Tom Walsh[3], Bill James[3], Jessica Monserrate[1], **Juan Ferré**[2] [1] Bayer U.S., Crop Science Division, 3500 Paramount Parkway, Morrisville, NC 27560 USA ; [2] ERI de Biotecnología y Biomedicina (BIOTECMED), Department of Genetics, Universitat de València, 46100 Burjassot, Spain ;[3]CSIRO, Black Mountain, Clunies Ross St., Acton, 2601, ACT Australia

BA-3 Characterization of a novel *Lysinibacillus sphaericus* myovirus vB_LspM-01 displaying pseudolysogeny

Peiling Geng, Xiaofu Wan, Shen Tian, Zhiming Yuan, **Xiaomin Hu** Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430070, China.

BA-4 Comparison of two carriers to formulate baits based on *Yersinia entomophaga* to control the African black beetle (*Heteronychus arator*)

Laura F. Villamizar[1], Marie Foxwell[1], David Wright[1], Sarah Mansfield[1], Jayanthi Swaminathan[1], Per Wessman[1,2], and Mark Hurst[1,] [1]AgResearch Ltd. Lincoln Research Centre. Christchurch 8140, New Zealand.[2]RISE Research Institutes of Sweden, Bioscience and Materials/Chemistry, Materials and Surfaces, Stockholm, Sweden

BA-5-STU Diversity and distribution of *cry* genes in *Bacillus* spp. strains using a universal PCR primer system and hidden Markov model profiles from the C-terminal end of Cry proteins.

J. Francisco Castillo-Esparza, Ismael Hernández-González, Javier Luévano-Borroel and Jorge E. Ibarra. CINVESTAV-Irapuato, Irapuato, Gto., Mexico

BA-6 Enriching and mining soil and grain metagenomes for novel insecticidal proteins

Irina Shilova[1], Amy Jo Johnson[1], Alex Gulevich[1], Ryan Dowdy[1], Sunit Jain[1], Paul Loriaux[1], Ryan J Williams[2], Ian W Davis[2], Jeff A Haas[2], Shoko Iwai[1], Prasanna Ramachandran[1], Erica Rutherford[1], Kim M Wegener[2], Thomas Weinmaier[1], Yonggan Wu[1], James Baum[2], Todd Z DeSantis[1], Karim Dabbagh[1], **Kristen Bennett**[11]Second Genome, United States, [2]Monsanto Co, United States

BA-7-STU Development of a Bacterial Pesticidal Protein Resource Center

Suresh Pannerselvam[1], Neil Crickmore[2], Colin Berry[3], Thomas Connor[3], Ruchir Mishra[1] and Bryony C. Bonning[11]Department of Entomology and Nematology, University of Florida[2]School of Life Sciences, University of Sussex[3]School of Biosciences, Cardiff University

BENEFICIAL INVERTEBRATES

DB-1-STU Artificial insemination techniques and sexually transmissible diseases in honey bees (*Apis mellifera*)

Thomas L. Gillard, Ben P. Oldroyd, Behaviour and Genetics of Social Insects Laboratory, School of Life and Environmental Sciences, The University of Sydney, Sydney, Australia

DB-2-STU Characterization of the major sensory organ and Ionotropic receptors of *Tropilaelaps mercedesae*

Jing Lei, Qiushi Liu and Tatsuhiko Kadowaki Department of Biological Sciences, Xi'an Jiaotong-Liverpool University, Suzhou, China

DB-3 Establishment of *Bacillus thuringiensis* based exogenous double-stranded RNA production platform

Jae Young Choi, Min Gu Park, Jong Hoon Kim, Dong Hwan ParK, Ra Mi Woo, Bo Ram Lee, Minghui Wang, Jun Young Kim, Yeon Ho Je Department of Agricultural Biotechnology, Seoul National University, Seoul, Republic of Korea

DB 4 Trypsin-mediated maturation of a novel antimicrobial peptide is important to resist bacterial infection in red swamp crayfish *Procambarus clarkii*

Yi Zheng[1], **Xian-Wei Wang**[1, 2] [1]Shandong Provincial Key Laboratory of Animal Cells and Developmental Biology, School of Life Sciences, Shandong University, Jinan, China; [2]State Key Laboratory of Microbial Technology, Shandong University, Jinan, China

DB-5-STU Impacts of change of environment on the composition of microbiota in Australian stingless bees, *Tetragonula carbonaria*.

Boyd Tarlinton, James McGree and Caroline Hauxwell, Queensland University of Technology (QUT), Brisbane 4000, Queensland, Australia

DB-6-STU Stress and the bee: the impacts of hive design and management practice on honey bees, *Apis melifera* L..

Daniel Cook, Caroline Hauxwell, James McGree and Thea Blackler Queensland University of Technology (QUT), Brisbane 4000, Queensland, Australia

DB-7-STU A new record of chalkbrood, *Ascosphaera solina*, isolated from and Australian native bee (*Amegilla cingulate*) of the Family *Apidae*

Nathaniel Crane & Caroline Hauxwell Queensland University of Technology (QUT), Brisbane 4000, Queensland, Australia

DB-8 Yeasts associated with nests of Australian stingless bees (*Meliponini*)

Flavia Massaro, **Lille Gill**, Boyd Tarlinton and Caroline Hauxwell, Queensland University of Technology, Invertebrate Microbiology Group, Science and Engineering Faculty, Gardens Point Campus, QLD 4001

DB-9-STU Characterization of the cross-interactions between Deformed Wing Virus (DWV), honey bee and the ectoparasitic mite, *Tropilaelaps mercedesae*

Yunfei. Wu[1], Xiaofeng Dong[2] and Tatsuhiko Kadowaki[11]Department of Biological Sciences, Xi'an Jiaotong-Liverpool University, Suzhou, China; [2]School of Life Sciences, Jiangsu Normal University, Xuzhou, China

FUNGI

FU-1-STU The lethality of *Beauveria bassiana s.l.* secondary metabolites on *Anopheles stephensi*

Yu Matsuzaki[1], Masanori Koike[2], Hirotaka Kanuka[3], Daigo Aiuchi[4] [1]Department of Animal Science and Agriculture, Obihiro University of Agriculture & Veterinary Medicine, Obihiro, Hokkaido, Japan; [2]Department of Agro-environmental Science, Obihiro University of Agriculture & Veterinary Medicine, Obihiro, Hokkaido, Japan; [3]Department of Tropical Medicine, The Jikei University School of Medicine, Tokyo, Japan; [4]Reserch Center for Global Agro-medicine, Obihiro University of Agriculture & Veterinary Medicine, Obihiro, Hokkaido, Japan

FU-2 Biology and control with the fungus *Metarhizium anisopliae* of *Demotispa neivai* (Coleoptera: Chrysomelidae) a pest of oil palm in Colombia

Luis Guillermo Montes Bazurto[1], Yimer Peteche Yonda[2], Alex Enrique Bustillo Pardey[11]Colombian Oil Palm Research Center (Cenipalma) [2]National University of Colombia

FU-3 Diversity of entomopathogenic fungi from Kintrishi National Area forest ecosystem of Georgia

Medea Burjanadze[1], Stefan Jaronski[2], Ketevan Koridze[1], Archil Supatashvili[11] Agricultural University of Georgia, Vasil Gulisashvili Fotest Institute, 0159 Tbilisi Georgia;[2] USDA ARS NPARL, 1500 N. Central, Ave., Sidney, MT,USA

FU-4 First records of *Beauveria* sp. and *Isaria* sp. occurrences in the invasive Brown Marmorated Stink Bug, *Halyomorpha halys* in Republic of Georgia

Medea Burjanadze[1], T.Abramishvili[2], Stefan Jaronski[3], Ketevan Koridze[1], Natalia kharabadze,[1] Vaja Vachadze[4],[1] Agricultural University of Georgia, Vasil Gulisashvili Fotest Institute, 0159 Tbilisi Georgia;[2]LMA,Laboratory of Ministry of Agricultural, 0159 Tbilisi, Georgia; [3] USDA ARS NPARL, 1500 N. Central, Ave., Sidney, MT,USA; [4]Medical State University of Georgia, Tbilisi, GEORGIA

FU-5-STU Potential of *Metarhizium* strains isolated in New Zealand against the grass grub (*Costelytra giveni*)

Nghia Nguyen, Travis Glare, Josefina Narciso and Michael Rostas, Bio-Protection Research Centre, Lincoln University, Lincoln, Christchurch, New Zealand

FU-6 Fungi *Metarhizium* spp. from Russia and neighboring territories: ecological preferences and activity against Colorado potato beetle larvae

Olga Yaroslavtseva[1], Vadim Kryukov[1], Oksana Tomilova[1], Maksim Tyurin[1], Evgeniy Elisaphenko[2], Yuriy Tokarev[3], Viktor Glupov [1,1]Institute of Systematics and Ecology of Animals SB RAS, Novosibirsk, Russia; [2]Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia; [3]All-Russia Institute of Plant Protection, St. Petersburg, Russia

FU-7 Successful field trial of *Metarhizium* (Hypocreales: Clavicipitacea) based mycoinsecticide for *Musca domestica* (Diptera: Muscidae) control in Australian cattle feedlots

Diana M. Leemon[1], **Dalton K. Baker**[1,2], Steven J. Rice[1], Rosamond M. Godwin[2], David G. Mayer[1], Peter J. James[2,1]Queensland Department of Agriculture and Fisheries, Ecosciences Precinct, Dutton Park, QLD 4102; [2]Queensland Alliance for Agriculture and Food Innovation, University of Queensland, St. Lucia, QLD 4072

FU-8 New mycopesticides for lesser mealworm (*Alphitobius diaperinus*) control in poultry houses

Steven J. Rice, Dalton K. Baker, Diana M. Leemon, Queensland Department of Agriculture and Fisheries, Ecosciences Precinct, Dutton Park, Qld 4102

FU-9 Influence of the plant hormone strigolactone on conidium germination and colonisation of plant roots by *Metarhizium anisopliae*

S. M. N. Islam & C. Hauxwell, Queensland University of Technology (QUT), Brisbane 4000, Queensland, Australia

FU-10 Effects of entomopathogenic fungi and a commercial bioinsecticide on brown marmorated stink bug, *Halyomorpha halys* (Stål) (Heteroptera: Pentatomidae) under laboratory conditions

Manana Kereselidze[1,3], **Andreas Linde**[2], Mzia Beruashvili[1] [1]Scientific Research Center of the Ministry of Agriculture**,** Tbilisi, Georgia;[2]Eberswalde University for Sustainable Development, Eberswalde, Germany;[3] V. Gulisashvili Forest Institute of the Agricultural University of Georgia

MICROSPORIDIA

MI-1-STU Molecular evidence of multiple microsporidian co-infections in mosquitos

Artur Trzebny[1], Anna Slodkowicz-Kowalska[2], Miroslawa Dabert,[1 1]Molecular Biology Techniques Laboratory, Faculty of Biology, Adam Mickiewicz University in Poznan, Poland; [2]Department of Biology and Medical Parasitology, Faculty of Medicine I, University of Medical Sciences, Poland

MICROBIAL CONTROL

MC-1 Pathogens and parasites of bark beetles and leaf-rolling weevils in Bulgaria

A. Linde[1], D. Takov[2] , D. Doychev[3], D. Pilarska [,2,4], S. Draganova[5], S. Nedelchev[6,1]Eberswalde University for Sustainable Development, Alfred-Möller-Straße, 16225 Eberswalde, Germany; [2]Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 1 Tzar Osvoboditel Str., Sofia, Bulgaria; [3]University of Forestry, 10 Kliment Ohridski Blvd., Sofia, Bulgaria;[4] Department of Natural Sciences, New Bulgarian University, 21 Montevideo Str., 1618 Sofia, Bulgaria;[5]Institute of Soil Science, Agrotechnologies and Plant Protection, 7 Shosse Bankya Str., Sofia, Bulgaria;[6]Department of Zoology and Anthropology, Sofia University, Dragan Tsankov Bvld., Bulgaria

MC-2-STU *Cajanus scarabaeoides* inhibits *Helicoverpa armigera* larvae development

Abigail Ngugi, Thi My Linh Hoang, Brett Williams, TJ Higgins and Sagadevan Mundree, Centre for Tropical Crops and Biocommodities, Queensland University of Technology, Brisbane, 4001, Australia

MC-3-STU Entomopathogenic fungi-mediated biological solution to control melon thrips *Thrips palmi*

Dongwei Li, Sihyeon Kim, Jong Cheol Kim, Se Jin Lee, Mi Rong Lee, So Eun Park, Sehyeon Baek, Tae Young Shin, Jae Su Kim, Department of Agricultural Biology, College of Agriculture & Life Sciences, Chonbuk National University, Korea

MC-4 The role of *Beauveria pseudobassiana* in restoration of Mana Island and a rare native Flax weevil*, Anagotus fairburni*

J. J. Brookes[1], T. R. Glare[1] and C. M. Miskelly[2 1]Bio Protection Research Centre, P. O Box 85084, Lincoln University, Lincoln, 7647, Christchurch, New Zealand, [2] Museum of New Zealand, Te Papa Tongarewa, Wellington, New Zealand

MC-5 Synergistic insecticidal activity of dsRNA specific to insulin signalling components with *Bacillus thuringiensis*

Yonggyun Kim Department of Plant medicals, Andong National University, Andong 36729, Korea

MC-6-STU Two new *Bacillus thuringiensis* strains toxic against *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

Maximiano C. Cassal[1], Shana F. Wiest[2], Chisa Yasunaga-Aoki[1], Lidia M. Fiuza[31]Laboratory of Insect Pathology and Microbial Control, Institute of Biological Control, Faculty of Agriculture, Kyushu University, Fukuoka, Japan; [2]Laboratory of Molecular Biology, Universidade do Vale do Rio dos Sinos (Unisinos), São Leopoldo, Brazil; [3]Rio-Grandense Rice Institute (IRGA), Cachoeirinha, RS, Brazil

MC-7 Characterization of chitinases of *Beauveria bassiana* (Bv062) induced in semisolid-state fermentation

Andrea Lovera[1,4], Mariano Belaich[2], Cindy Mejía[1], Laura Villamizar[3], Manuel Patarroyo[4], **Gloria Barrera[1,]** [1]Corporación Colombiana de Investigación Agropecuaria - AGROSAVIA, Centro de investigación Tibaitatá – Km 14 vía Mosquera - Bogotá, Colombia. [2]Laboratorio de Ingeniería Genética y Biología Celular y Molecular-Área Virosis de Insectos, Universidad Nacional de Quilmes, Provincia de Buenos Aires, Argentina. [3]AgResearch Ltd. Lincoln Research Centre. Christchurch 8140, New Zealand. [4]Universidad Nacional de Colombia. [4]Fundación Instituto de Inmunología de Colombia-FIDIC

MC-8 In-plant protection from the insect pest *Helicoverpa armigera* by trans-kingdom RNAi

Julia Bally[1], Karen Lee[1], Samanta Bolzan de Campos[1], Marcelo German[2], Elane Fishilevich[2], Rachel L. Doran[1], Kenneth E. Narva[2] and Peter M. Waterhouse,[11] Centre for Tropical Crops and Biocommodities, QUT, Brisbane, QLD, Australia

MC-9-STU Chitinase of *Trichoderma koningiopsis* to enhance the insecticidal activity of *Beauveria bassiana* to control the sugar cane borer *Diatraea saccharalis*

Cindy Mejía[1], Harold Ardila[2], Carlos Espinel[1], Pedro F. B. Brandão [2], Laura Villamizar,[3 1]Corporación Colombiana de Investigación Agropecuaria - AGROSAVIA, Centro de investigación Tibaitatá - Km 14 vía Mosquera – Bogotá, Colombia; [2]Universidad Nacional de Colombia, Bogotá, Colombia; [3]AgResearch Ltd. Lincoln Research Centre. Christchurch 8140, New Zealand

MC-10-STU Evaluation of additives to induce enzymatic activity of *Beauveria bassiana* conidia and improve insecticidal activity against *Diatraea saccharalis*

Cindy Mejía[1], Carlos Espinel[1], Pedro F. B. Brandão[2], Laura Villamizar[3, 1]Corporación Colombiana de Investigación Agropecuaria - AGROSAVIA, Centro de investigación Tibaitatá - Km 14 vía Mosquera - Bogotá, Colombia; [2]Universidad Nacional de Colombia, Sede Bogotá, Departamento de Química; [3]AgResearch Ltd. Lincoln Research Centre. Christchurch 8140, New Zealand

MC-11-STU Selection of an adequate substrate to produce high quality conidia with a Colombian isolate of *Beauveria bassiana* to control the sugar cane borer *Diatraea saccharalis*

Cindy Mejía[1], Carlos Espinel[1], Mateo Forero[2], Freddy A. Ramos[2], Pedro F. B. Brandão[2], Laura Villamizar,[3] [1]Corporación Colombiana de Investigación Agropecuaria - AGROSAVIA, Centro de investigación Tibaitatá - Km 14 vía Mosquera - Bogotá, Colombia; [2]Universidad Nacional de Colombia, Sede Bogotá, Departamento de Química; [3]AgResearch Ltd. Lincoln Research Centre. Christchurch 8140, New Zealand.

MC-12 Evaluation and monitoring of maize crops in Brazil expressing the Cry1F toxin from *Bacillus thuringiensis* in the control of *Helicoverpa armigera* and *Spodoptera frugiperda*

Cristina Lima de Macedo[1], **Elias Ferreira Sabiá Júnior[2]**, Briana Cardoso Ferreira[1], Érica Soares Martins[3], Paulo Roberto Martins Queiroz[3], Rose Gomes Monnerat[1,]1Embrapa Recursos Genéticos e Biotecnologia, Brasília, Brasil; [2]Instituto de Ciências Biológicas, Universidade de Brasília, Brasília, Brasil; [3]Instituto Mato-grossense do Algodão, Mato Grosso, Brasil

MC-13-STU Identification and characterization of novel juvenile hormone antagonists from *Streptomycetes*

Bo Ram Lee, Dong Hwan Park, Jae Young Choi, Jong Hoon Kim, Min Gu Park, Jun Young Kim, Minghui Wang, Yeon Ho Je, Department of Agricultural Biotechnologh, Seoul National University, Seoul, Republic of Korea

MC-14 Microbial control of the western grapeleaf skeletonizer

Suchitra S. Dara[1,2], Alor Sahoo[2], Surendra K. Dara[3], Stefan T. Jaroski[4, 1]Global Agricultural Solutions, Bakersfield, CA, USA; [2]Stockdale High School, Bakersfield, CA, USA; [3]University of California Cooperative Extension, San Luis Obispo, CA, USA; [4]USDA-ARS, Sidney, MT, USA

MC-15 Selection and Characteristics of Entomopathogenic Fungi for Microbial Control of *Plutella xylostella*

Ji Hee Han, Jae Yoon Kim, Moran Lee, Hye Ju Jeong, Dayeon Kim, Seongho Ahn and Sang Yeob LeeAgricultural Microbiology Division, National Institute of Agricultural Sciences, RDA, Wanju, 55365, Republic of Korea

MC-16 Entomopathogenic Fungi for Dual Control of Thrips and Plant Fungal Disease

Ji Hee Han, Moran Lee, Jae Yoon Kim, Hye Ju Jeong, Dayeon Kim, Seung Ho Ahn, and Sang Yeob LeeAgricultural Microbiology Division, National Institute of Agricultural Sciences, RDA, Wanju, 55365, Republic of Korea

MC-17-STU Laboratory and field evaluation of entomopathogenic fungi and bacteria for the control of *Rachiplusia nu* (Guenée) (Lepidoptera: Noctuidae) in soybean (*Glycine max* (L.) Merrill).

Matías S. Abalo[1], Sebastian A. Pelizza[2,3], Ana C. Scorsetti[2]. [1]Rizobacter Argentina S.A. [2]Instituto de Botánica Carlos Spegazzini, FCNyM-UNLP, La Plata, Buenos Aires, Argentina, [3]Centro de Estudios Parasitológicos y de Vectores (CEPAVE), CCT La Plata-CONICET-UNLP, La Plata, Buenos Aires, Argentina

NEMATODES

NE-1-STU Diversity of entomopathogenic nematodes and their symbiotic bacteria in Australian soils and their interaction with Queensland fruit fly (*Bactrocera tryoni*)

Sitaram Aryal[1], Uffe N. Nielsen[1], Markus Riegler[1] [1]Hawkesbury Institute for the Environment, Western Sydney University, Locked Bag 1797, Penrith, NSW 2751, Australia

NE-2 Isolation of cell wall encapsulated or purified Cry5B crystals from asporogenous *Bacilli* for use as a anthelminthic drug

Ambily Abraham, Deysy Tatiana Pinto Rodriguez, Yan Hu, Hanchen Li, Kelly Flanagan, David Gazzola, Tasia Kellogg, Gary Ostroff and Raffi Aroian Program of Molecular Medicine, University of Massachusetts Medical School, Worcester, MA, 01605, USA

NE-3 Characterization of the heat shock protein 90 gene of *Heterorhabditis bacteriophora* and its expression in response to different temperature stress.

Elena Fanelli[1], Giuseppina Moscatelli[1], Alberto Troccoli[1], Monica Oreste[2], **Eustachio Tarasco**[1,2], Francesca De Luca[11]Istituto per la Protezione Sostenibile delle Piante (IPSP), CNR, Via Amendola 122/D, 70126 Bari, Italy; [2]Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi di Bari "Aldo Moro", Via Amendola 165/a, 70126 Bari, Italy

NE-4 Trait derioration rate of entomopathogenic nematodes and selection of superior inbred lines

Tshima Ramakuwela[1], Barbara L. Caoili [2], David I. Shapiro-Ila[3], [11]Agricultural Research Council-Small Grains (ARC-SG), Bethlehem, South Africa; [2]Institute of Weed Science, Entomology and Plant Pathology, College of Agriculture and Food Science U.P. Los Baños, Philippines; [3]USDA-ARS, South Eastern Fruit and Tree Nut Research Laboratory, Byron, USA

NE-5-STU Genetic diversity of the biocontrol nematode *Deladenus siricidicola* in Australia and New Zealand

Firehiwot B. Eshetu[1], Helen Nahrung[2], Irene Barnes[1], Katrin N.E. Fitza[1], Stephen Elms[3], and Bernard Slippers[1*1]Department of Biochemistry, Genetics and Microbiology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, 0002, South Africa; **[2]**Forest Industries Research Centre, University of the Sunshine Coat, Queensland, Australia; [3]HVP Plantations, Churchill, Victoria, Australia

NE-6-STU Improvement of oxidative stress tolerance and longevity of the entomopathogenic nematode *Heterorhabditis bacteriophora* through genetic selection

Nanette Hope Sumaya[1,2], Bart Vandenbossche[1], Mike Barg[1], Verena Doerfler[1], Olaf Strauch[1], Carlos Molina[1] and Ralf-Udo Ehlers [1,2] [1]e–nema, GmbH, Klausdorfer Str. 28-36, 24223 Schwentinental, Germany; [2]Faculty of Agricultural and Nutritional Sciences, Christian-Albrechts-University of Kiel, Kiel, Germany

NE-7 A survey of the parasitic nematodes of invertebrates in Mindanao Island, the Philippines

Elena S. Ivanova[1]., Malysheva, S. [1], **Sumaya, N.H.N.**[2], Mohagan, A.[3] and Spiridonov, S.E. [1 1]Centre of Parasitology, A.N. Severtsov's Institute of Ecology and Evolution, Russian Academy of Sciences, Leninskii prospect, 33, Moscow, 119071, Russian Federation;[2] Department of Biological Sciences, College of Science and Mathematics, Mindanao State University-Iligan Institute of Technology, A. Bonifacio Ave., Tibanga, Iligan City, the Philippines; and [3] Department of Biological Sciences, Central Mindanao University, Musuan, Bukidnon, the Philippines

NE-8 Entomopathogenic nematode, *Steinernema kraussei* – the first recorded from Korea and temperature effect on Ulleungdo strain

Young Hak Jung[1], Ho Yul Choo[,2], **DongWoon Lee[3]** [1]SM Biovision Co. Ltd., Jinju, Gyeongsangnam-do, Republic of Korea; [2]Emeritus Professor of Gyeongsang National University and Managing Consultant of Nambo Co. Ltd., Jinju, Gyeongsangnam-do, Republic of Korea; [3]Major of Applied Biology, School of

Ecological Environment and Tourism, Kyungpook National University, Sangju, Gyeongsangbuk-do, Republic of Korea

NE-9 Slug parasitic nematode presence in Delaware

Ivan Hiltpold[1], Bill Cissel[1], Brian Kunkel[1] [1]Department of Entomology and Wildlife Ecology, College of Agriculture, University of Delaware, Newark, DE, USA

NE-10 Cloning, expression and insecticides activity of ATP binding protein in *Aedes aegypti* against Bt

Guohui Zhao, Liannan Liu, Xiaohua Hu, Xiong Guan and **Lingling Zhang** State Key Laboratory of Ecological Pest Control for Fujian and Taiwan Crops, Life of Science, Fujian Agriculture and Forestry University, 350002 Fuzhou, Fujian, People's Republic of China

VIRUSES

VR-1 Baculoviruses as a tool for generating stable and effective sub-unit vaccines

Mine Aksular[1,2], Adam Chambers[1], Robert D Possee[1,2], Eva Calvo-Pinilla[3], Javier Ortego[3], Javier Castillo-Olivares[2], Linda A King[,2] [1]Oxford Expression Technologies Ltd, Oxford, UK, [2]Department of Biological and Medical Sciences, Oxford Brookes University, Oxford, UK, [3]CISA-INIA, Valdeolmos, Madrid, Spain

VR-2 Biological and genetic patterns of *Lymantria dispar* multiple nucleopolyhedrovirus strain with cubic shape of occlusion bodies

Vyacheslav Martemyanov[1], Sergey Pavlushin[1], Yury Ilinsky[2], Evgeny Lunev[3], Stepan Toshchakov[3,4] [1]Institute of Systematics and Ecology of Animals SB RAS, Laboratory of Ecological Parasitology, Novosibirsk, Russia; [2]Institute of Cytology and Genetics SB RAS, Laboratory of Molecular Genetics of Insects, Novosibirsk, Russia;[3]Immanuel Kant Baltic Federal University, Kaliningrad, Russia; [4]Winogradsky Institute of Microbiology, Research Centre of Biotechnology, RAS, Moscow, Russia

VR-3 Development and characterisation of BacMAM vectors for expression of protective genes in pancreatic islet tissue: towards a therapy for Diabetes type 1 in Mexico

Leo Graves[1,2], Mine Aksular[1], Daniel Ruiz Buck[1], Adam Chambers[1], Stephen Hughes[3], Paul Johnson[3], Juanjose Plata-Munoz[4], Fernanda Murguia-Meca[4], Riyadh Abdulsahib[2], Robert Possee[1,2] and Linda King[2] [1]Oxford Expression Technologies Ltd, Oxford, UK, [2]Department of Biological and Medical Sciences, Oxford Brookes University, Oxford UK, [3]Oxford Consortium for Islet Transplantation, Nuffield Department of Surgical Sciences, University of Oxford, Oxford UK, [4]Centre for Molecular and Cell-Based Therapeutics, Mexico City, Mexico

VR-4 Development of BacMAM vectors to improve transduction efficiency of mammalian cells

Adam Chambers[1], Leo Graves[1,2], Mine Aksular[1], Daniel Ruiz Buck[1], Linda A King[2], Robert D Possee[1,2] [1]Oxford Expression Technologies Ltd, Oxford, UK, [2]Department of Biological and Medical Sciences, Oxford Brookes University, Oxford UK

VR-5-STU Display of surface protein by baculovirus for improving the stability of influenza virus hemagglutinin through structure-guided motif swapping

Chih-Hsuan Tsai, Yu-Chan Chao Institute of Molecular Biology, Academia Sinica, Taipei 115, Taiwan, ROC

VR-6-Host miRNAs are involved in hormonal regulation of HaSNPV-triggered climbing behavior in *Helicoverpa armigera*

Songdou Zhang[1], Shiheng An[2], Zhen Li[1]**, Xiaoxia Liu**[1, 1] Department of Entomology, China Agricultural University, Beijing, 100193, China [2]College of Plant Protection, Henan Agricultural University, Zhengzhou, 450002, China

VR-7 Identification of miRNAs and target genes associated with to codling moth resistance against Cydia pomonella granulovirus

Yu Xi[1], Cong Huang[2], Qiang Wu[2], Johannes A. Jehle[3], Fanghao Wan[1,2] [1]Agricultural Genomes Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen, China; [2]Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China; [3]Institute for Biological Control, Julius Kühn-Institut, Darmstadt, Germany

VR-8 New method for granulovirus differentiation based on real-time PCR

Martyna Krejmer-Rabalska[1], Lukasz Rabalski[1], Marlinda Lobo de Souza[2], Sean Moore[3,4], Michael D. Jukes[4], Boguslaw Szewczyk[1] [1] Department of Recombinant Vaccines, Intercollegiate Faculty of Biotechnology of the University of Gdansk and Medical University of Gdansk, Gdansk, Poland [2]*Embrapa Recursos Genéticos e Biotecnologia, , Brasilia, Brazil* [3]Citrus Research International (CRI), Port Elisabeth, South Africa [4]Centre for Biological Control, Department of Biochemistry and Microbiology, Rhodes University, Grahamstown, South Africa

VR-9 Novel virus hunting in Australian *tephritid* fruit flies

Stephen R Sharpe[1], Jennifer L Morrow[1], Alexie Papanicolaou[1], Toni A Chapman[2], James M Cook[1], Markus Riegler[1] [1]Hawkesbury Institute for the

Environment, Western Sydney University, Locked Bag 1797, Penrith, NSW 2751, Australia; [2]NSW Department of Primary Industries, Elizabeth Macarthur Agricultural Institute, Woodbridge Road, Menangle, NSW 2568, Australia.

VR-10 Pathogenicity and genome sequence of an isolate of Lymantria dispar multiple nucleopolyhedrovirus from China

Robert L. Harrison[1], Daniel L. Rowley[1], Melody A. Keena[2] [1]Invasive Insect Biocontrol and Behavior Laboratory, Beltsville Agricultural Research Center, USDA Agricultural Research Service, 10300 Baltimore Avenue, Beltsville, Maryland, USA; [2]Northern Research Station, USDA Forest Service, 51 Mill Pond Road, Hamden, CT, USA

VR-11 Visualization of different protein maturation based on their baculovirus transmembrane signal.

Maciej Kosiński[1],Natalia Derewońko[2], Martyna Krejmer-Rąbalska[1], Aurelia Schweda[1], Łukasz Rąbalski[1], Bogusław Szewczyk[1] [1]Laboratory of Recombinant Vaccines, Intercollegiate Faculty of Biotechnology of the University of Gdańsk and Medical University of Gdańsk, Gdańsk, Poland; [2]Laboratory of Virus Molecular Biology, Intercollegiate Faculty of Biotechnology of the University of Gdańsk and Medical University of Gdańsk, Gdańsk, Poland

VR-12-STU *Pe38* is not the only gene that responds to type I resistance in codling moth (*Cydia pomonella* L.)

Jiangbin Fan [1,2], Jörg T. Wennmann [1], Dun Wang [2], Johannes A. Jehle [1] [1]Julius Kühn-Institut, Federal Research Centre for Cultivated Plants, Heinrichstraße 243, 64287 Darmstadt, Germany; [2]Key Laboratory of Plant Protection Resources and Pest Management of Ministry of Education, Northwest A&F University, Yangling 712100, China

VR-13 Confirmation of two zinc-binding domains in *baculovirus* protein ME53 and its association with the ribosome during Autographa californica multiple *nucleopolyhedrovirus* infection

Robyn Ralph and **Peter Krell;** Department of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario, Canada

VR-14 Interaction of VP80 and ME53 from Autographa californica nucleopolyhedrovirus

Emine Özşahin[1], Éva Nagy[2], Daniel Doucet[3] and **Peter J. Krell[1];** [1]Department of Molecular and Cellular Biology, University of Guelph, Ontario, Canada; [2]Department of Pathobiology, University of Guelph, Ontario, Canada, [3]Natural Resources Canada, Sault Ste Marie, Ontario, Canada

VR-15 Advances in the use of *CRISPR/Cas* technology to edit baculovirus genomes

Nugnes, María Victoria; Ghiringhelli, Pablo Daniel; **Belaich, Mariano Nicolás**. Laboratory of Genetic Engineering and Cellular and Molecular Biology, Area of insect viruses (LIGBCM-AVI); Institute of Basic and Applied Microbiology (IMBA); Universidad Nacional de Quilmes. Roque Saenz Peña 352, Bernal (Buenos Aires), Argentina

VR-16 AcMNPV-miR-2 facilitates AcMNPV infection by down-regulating the expression of viral own genes and host immune related proteins

Xinghua Yu, **Jinwen Wang**, Xunzhang Wang School of Life Sciences, Sun Yat-Sen University, Guangzhou 510275, China

VR-17 Characterization of *Autographa californica Nucleopolyhedrovirus ac75* and its role in the morphogenesis of budded virions and occlusion-derived virions

Anqi Shi, Zhaoyang Hu, Yachao Zuo, Yan Wang, Wenbi Wu, **Meijin Yuan**, Kai Yang State Key Laboratory of Biocontrol, Sun Yat-sen University, Guangzhou 510275, China

VR-18 Determination of *Anticarsia* MNPV and *Spodoptera* MNPV co-infection in insect cell culture

Giovana C. Guimarães[1,2], William Sihler[1], Marlinda L. Souza[1], **Márcio M. Sanches[1]** [1]Embrapa Recursos Genéticos e Biotecnologia, Brasília, Brasil; [2]Centro Universitário de Brasília, Brasília, Brasil

VR-19 Functional studies on the host AAA+ ATPase Ter94 in baculovirus life cycle

Yimeng Li[1,2], Liangbo Hu[1,2], Tong Chen[1,2], Meng Chang[1,2], Fei Deng[1], Zhihong Hu[1], Manli Wang[1*], **Hualin Wang**[1*] [1]State Key Laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, P.R. China;[2]University of Chinese Academy of Sciences, Beijing 100049, P. R. China

VR-20 The conserved amino acid N27 of baculovirus Ac110 is important for oral infection

Leyuan Zhu[1], Jiantao Liu[2], Yanling Wang[1], Meijin Yuan[1], **Wenbi Wu**[1], Kai Yang[1]

[1]State Key Laboratory of Biocontrol, Sun Yat-sen University, Guangzhou 510275, China

[2]Jiangxi Key Laboratory of Bioprocess, Jiangxi Science & Technology Normal University, Nanchang, Jiangxi, 330013, China

VR-21 Construction of Baculovirus inducible expression system for rapid development of virus like particle vaccines

WonSeok Gwak[1], Hyun Soo Kim[1], SooDong Woo[1]
[1]Department of Agricultural Biology, College of Agriculture, Life & Environment Science, Chungbuk National University, Cheongju 28644, Korea

15.30-16.00 AFTERNOON TEA

Contributed papers Pipeline
Wednesday 16.00-18.00

BACTERIA 2

Moderator: O. P. Perera

16.00 **102** **Alkaline adaptation of *Bacillus thuringiensis* regulated by Crp protein**
Zhongqin Sun[1], Fan Yang[2], Guiwei Kao[2], Tantan Gao[2], Qi Peng[2], Jie Zhang[2], **Shuyuan Guo**[1], Fuping Song[2] [1]School of Life Science, Beijing Institute of Technology, Beijing 100081, China[2] State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China

16.15 **103-STU** **Characterization of the resistance to *Bacillus thuringiensis* Vip3Aa protein in a *Heliothis virescens* laboratory population**
Daniel Pinos[1], Anabel Millán-Leiva[1], Juan Luis Jurat-Fuentes[2], Dennis J. Wright[3], Patricia Hernández-Martínez[1], Juan Ferré[1] [1]ERI of Biotechnology and Biomedicine (BIOTECMED), Universitat de València, Burjassot, Spain.[2]Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, USA.[3]Department of Life Sciences, Faculty of Natural Sciences, Imperial College London, Berkshire, UK

16.30 **104** **Defining the virulence determinants of *Serratia proteamaculans AGR96X* and its capacity for protection of establishing ryegrass from larvae of New Zealand grass grub *(Costelytra giveni)* and manuka beetle *(Pyronota* spp.*).***
Mark RH Hurst[1,2]; Amy Beattie[1]; David Wright[1], Sandra Young[1], Chikako van Koten[1] and Maureen O'Callaghan[1,2] AgResearch, Lincoln Research Centre, Private Bag 4749, Christchurch 8140, New Zealand, [2]Bio-Protection Research Centre, Lincoln, Christchurch, New Zealand

16.45 **105** **Two strong promoters for *cry* gene expression**
Xin Zhang[1, 2], Tantan Gao[1], Lixin Du[3], Qi Peng[1], Jie Zhang[1], Dongmei Sun[2], **Fuping Song**[1]* [1]State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China; [2]College of Life Science and Technology, Heilongjiang Bayi Agricultural University, Daqing 163319, China; [3]Institute of Plant Protection, Hebei Academy of Agricultural and Forestry Sciences, Baoding 071000, China

17.00 **106** **Bt or not Bt? Genome analysis of mosquitocidal *Bacillus wiedmannii* biovar *thuringiensis* strain FCC41**
J. Nicolás Lazarte, Rocio P. Lopez, **Corina M. Berón** Instituto de Investigaciones en Biodiversidad y Biotecnología (INBIOTEC) - CONICET, FIBA, Mar del Plata - Argentina.

17.15 **107** **Examining putative insecticidal toxins from the bacterium *Brevibacillus laterosporus***
Marsha Ormskirk[1], Travis Glare[1], John Hampton[1], Santanu Deb Choudhury[2], James Vernon[2], Fariba Nourozi[1] and Jason Busby[31] Bio-Protection Research Centre, PO Box 85084, Lincoln University, Lincoln 7647, New Zealand. [2] AgResearch Limited, Agriculture Research Centre, Private bag 4749, Christchurch 8140, New Zealand. [3]Auckland University, Private bag 92019, Auckland 1142, New Zealand

17.30 **108** Cancelled

Contributed papers Maui 3
Wednesday 16.00-18.00

Beneficial Invertebrates & Viruses 2

Moderator: Lyric Bartholomay and Kelly Bateman

16.00 **109** **Histone deacetylase inhibitor-treatment restores memory-related gene expression and learning ability in neonicotinoid-treated *Apis mellifera***
Yee-Tung Hu, **Cheng-Kang Tang**, Carol-P Wu, Pei-Chi Wu, En-Cheng Yang, Chia-Chi Tai and Yueh-Lung Wu Department of Entomology, National Taiwan University, Taipei 106, Taiwan

16.15 **110** **Coral, photosynthesis, and the emergence of parasitism in *Apicomplexa***
Patrick J. Keeling Botany Department, University of British Columbia

16.30 **111-STU** **Cracking the code of Pacific oyster mortality syndrome**
Aude Lucasson[1,2],Julien de Lorgeril[1], Bruno Petton[3,4], Eve Toulza[5], Caroline Montagnani[1], Camille Clerissi[1,6], Jeremie Vidal-Dupiol[1], Cristian Chaparro[6], Richard Galinier[6], Jean-Michel Escoubas[7] Philippe Haffner[1], Lionel Degremont[8], Guillaume M. Charrière[2], Maxime Lafont[1,5], Abigaël Delort[1], Agnès Vergnes[1], Marlène Chiarello[9], Tristan Rubio[2], Marc Leroy[7], Adeline Pérignon[10], Denis Régler[10], Marianne Alumno-Bruscia[3,4], Pierre Boudry[3,11], Frédérique Le Roux[3,12], Delphine Destoumieux-Garzón[7], Yannick Gueguen[1], Guillaume Mitta[5] . France

16.45 **112** **Honey Bees in Peril: The use of cricket paralysis virus as a model honey bee virus system to study colony collapse disorder**
Carol Fassbinder-Orth, Ryan Sabotin, Tammy Tran Creighton University, Omaha, NE, USA

17.00 **113 Divergence from the PDV paradigm in the repeated evolution of associations between mutualistic viruses and parasitoid wasps**
Gaelen R. Burke, Kelsey A. Coffman
Department of Entomology, the University of Georgia, Athens, Georgia, USA

Contributed papers Maui 1 & 2
Wednesday 16.00-18.00
Fungi 2
Moderator: Komivi Akutse

16.00 **114 Germination of *Beauveria pseudobassiana* microsclerotia and biocontrol activity against the African black beetle (*Heteronychus arator*)**
Laura F. Villamizar[1], Gloria P. Barrera[2], Marie Foxwell[1], Sean D.G. Marshall[1], Marina Richena[1], Duane Harlan[1], Trevor A. Jackson[1 1]AgResearch Ltd. Lincoln Research Centre. Christchurch 8140, New Zealand. [2]Universidad Nacional de Colombia. [2]Corporación Colombiana de Investigación Agropecuaria - Corpoica, Centro de investigación Tibaitatá – Km 14 vía Mosquera - Bogotá, Colombia.

16.15 **115 *Leptoglossus occidentalis*** (Heidemann, 1910)**, an invasive species attacking conifers in Lebanon: preliminary laboratory control by the entomopathogen *Beauveria bassiana***
Yara El Khoury [1,2], Elise Noujeim[1], **Eustachio Tarasco**[2], Nabil Nemer[3] [1]National Center for Marine Sciences, National Council for Scientific Research -CNRS, P.O.Box 11-8281, Ryad El Solh 1107 2260, 59, Zahia Selman Street, Beirut, Lebanon; [2]Department of Soil, Plant and Food Sciences, Section of Entomology and Zoology, University of Bari 'A. Moro, Bari, Italy; [3]Holy Spirit University of Kaslik, Faculty of Agricultural and Food Sciences, PO Box 446, Jounieh, Lebanon

16.30 **116 Wheat growth-response to endophytic *Beauveria bassiana* following fungal encounters from insect versus plant sources**
Lisemelo F. Motholo[1, 2], Mardé Booyse[3], Justin L. Hatting[a], Toi J. Tsilo[a], Oriel M. M. Thekisoe[2].
[1] Agricultural Research Council – Small Grain, Private Bag X29, Bethlehem, 9700, South Africa; [2] Unit for Environmental Sciences and Management, North-West University, Private Bag X6001, Potchefstroom, 2520, South Africa; [3]Agricultural Research Council – Biometry, Private Bag X5013, Stellenbosch, 7599, South Africa

16.45 **117-STU Elucidating the natural function of cordycepin, a metabolite of the fungus *Cordyceps militaris***
Victoria Woolley, Graham Teakle and Dave Chandler Warwick Crop Centre, School of Life Sciences, University of Warwick, Wellesbourne, Warwick, U.K

17.00 **118 Combined utilization of *Beauveria bassiana* and spinosad against wireworms *Agriotes lineatus* and *Agriotes obscurus* (Coleoptera: Elateridae).**
Pierre-Antoine Bourdon[1], Ian Baxter[2] and Tariq Butt[1 1] Biocontrol and Natural Products, Swansea University, Wales, United Kingdom; [2]Certis Europe, Maarssen, The Netherlands

17.15 **119 Multigene systematics and ecological associations of two proposed new species of *Metarhizium* from Australia.**
S. M. N. Islam, T. Scharaschkin & C. HauxwellQueensland University of Technology (QUT), Brisbane 4000, Queensland, Australia

20.00-22.00 Maui 1 & 2
DBI Division Business Meeting

20.00-22.00 Maui 3
Fungi Division Business Meeting

THURSDAY 16 August 2018

Fungal Division Symposium Pipeline
Thursday 08.00-10.00
Fungus-insect interactions in post genomic era: Advances and perspectives - Genomic and transcriptomic studies on *Beauveria* including plant associations
Organiser/Moderator: Chengshu Wang and Jae Su Kim

8.00 **120 Genomic and transcriptomic studies on *Beauveria* including plant associations**
Travis R. Glare[1], Maya Raad[1], Aimee C. McKinnon[1], Maria E. Moran-Diez[2], Peter C.H. Cheong[3], Michael Rostas[1 1] Bioprotection Research Centre, Lincoln University, Lincoln, New Zealand; [2] Universidad de Salamanca, Salamanca, Spain, [3]Molecular Medicine, Faculty of Medicine, University of Malaya, Malaysia

8.30 **121** Cancelled

9.00 **122 Genetic analysis of *Beauveria bassiana* JEF-007 as a biopesticide against bean bug**
Se Jin Lee, Sihyeon Kim, Mi Rong Lee, Jong Cheol Kim, So Eun Park, Tae Young Shin, Baek Sehyeon, Jae Su Kim. Department of Agricultural Biology, College of Agriculture & Life Sciences, Chonbuk National University, Korea

9.30 **123** Cancelled.

Contributed papers Maui 1 & 2
Thursday 8.00-10.00

Viruses 4

Moderators: Abd-Alla A.M.M. and Chejanovsky Nor

8.00 **124** **Mechanism of salivary gland hypertrophy virus (SGHV) infections: Prerequisite for tsetse and trypanosomosis control**
Meki, I.K.[1, 2], Kariithi, H.M.[1, 3], Parker, A.G.[1], Vreysen M.J.B.[1], Ros, V.I. [2], Vlak, J.M.[2], van Oers, M.M.[2] and **Abd-Alla A.M.M**.[11] Insect Pest Control Laboratory, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, International Atomic Energy Agency, Vienna, Austria.[2] Laboratory of Virology, Wageningen University and Research, 6708 PB Wageningen, The Netherlands [3] Biotechnology Research Institute, Kenya Agricultural and Livestock Research Organization, P.O Box 57811, Loresho, Nairobi, Kenya

8.15 **125-STU** **Deciphering the population structure of genotype mixtures of CpGV field isolates by next generation sequencing techniques and improved sequence analyses methods**
Jiangbin Fan [1,2], Jörg T. Wennmann [1], Dun Wang [2], Johannes A. Jehle [1] [1]Julius Kühn-Institut, Federal Research Centre for Cultivated Plants, 64287 Darmstadt, Germany; [2]Key Laboratory of Plant Protection Resources and Pest Management of Ministry of Education, Northwest A&F University, Yangling 712100, China

8.30 **126** **Construction of a reverse genetics system of *Dendrolimus punctatus cypovirus* and its application**
Gaobo Zhang, Congrui Xu, Jia Wang, Jian Yang, Chengfeng Lei, Jia Hu, **Xiulian Sun** Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, 430071, Hubei, China

8.45 **127-STU** **Developmental resistance to natural infection by DCV in *Drosophila melanogaster***
Simon Villegas-Ospina, Karyn N. Johnson. School of Biological Sciences, Faculty of Science, The University of Queensland, Brisbane, Australia

9.00 **128** **Identification of Osugroshi virus, a late male-killing virus in *Homona magnanima***
Ryosuke Fujita[1,2], Maki Inoue[3], Takumi Takamatsu[3], Hiroshi Arai[3], Mayu Nishino[3], Hironori Koyama[3], Nobuhiko Abe[3], Kentaro Itokawa[4], Madoka Nakai[3], and Yasuhisa Kunimi[3] [1]Isotope Imaging Laboratory, Creative Research Institution, Hokkaido University, Sapporo, Japan, [2]Department of Medical Entomology, National Institute of Infectious Diseases, Tokyo, Japan, [3]Department of Applied Biological Science, Tokyo University of Agriculture and Technology, Saiwaicho, Fuchu, Tokyo, Japan, [4]Department of Medical Entomology, National Institute of Infectious Diseases, Tokyo, Japan.

9.15 **129** **Replication of Apis Rhabdovirus-1\Bee Rhabdovirus-1, a negative-sense RNA virus of pollinators, in *Apis mellifera* and *Varroa destructor***
Levin Sofia[1,2], Galbraith David[3], Sela Noa[4], Erez Tal[1], Grozinger Christina M[3] and **Chejanovsky Nor**[1] [1]Entomology Department, Institute of Plant Protection, Agricultural Research Organization, Israel, [2]Faculty of Agricultural, Food and the Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot, Israel, [3]Department of Entomology, Center for Pollinator Research, Huck Institutes of the Life Sciences, Pennsylvania State University, University Park, PA, [4] Department of Plant Pathology and Weed Research, Institute of Plant Protection, Agricultural Research Organization, Israel

9.45 **130** **Cryo-EM structure reveals cylindrical nucleocapsids from two Polydnaviruses**
Ji-Hui Cui[1,2,3,†], Ya-Bin Chen[1, 3,†], Ming Li[1,2,3,†], Qiu-Chen Cai[1,2,3], Li-Dan Zhang[1,2,3], Zi-Yun Lu[4], Jian-Cheng Li[4], Qi-Shun Zhu[1,3], Gang Ji[5,*], **Kai-Jun Luo**[1,2,3,*] [1]School of Life Sciences, Yunnan University, Kunming, P.R. China; [2]Key Laboratory for Biochemistry and Molecular Biology of High Education in Yunnan Province, Yunnan University, Kunming, P.R. China; [3] Key Laboratory for Animal Genetic Diversity and Evolution of High Education in Yunnan Province, Yunnan University, Kunming, P.R. China; [4]Institute of Plant Protection, Hebei Academy of Agricultural and Forestry Sciences, Baoding, Hebei 071000, P.R. China;[5] National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, 15 Datun Road, Beijing 100101, P.R. China

Contributed papers — Maui 3

Thursday 8.00-10.00

Microbial Control 3

Moderator: Mary Babercheck

8.00 **131-STU** **Laser Capture Microdissection to study iron homeostasis gene expression in *Bacillus cereus* during *Galleria mellonella* midgut infection**
Laurent Consentino[1], Agnès Réjasse[1], Nicolas Crapart[2,3], Christophe Buisson[1], Claudia Bevilacqua[2], Christina Nielsen-Leroux[1] [1] INRA, UMR 1319 MICALIS & AgroParisTech, 78350 Jouy-en-Josas, France; [2] INRA, UMR 1313 GABI, plateforme @BRIDGE, 78350 Jouy-en-Josas, France; [3] Excilone, 6-10 rue Blaise Pascal, 78990 Elancourt, France.

8.15 **132** **Plant functional traits, but not diversity, and soil characteristics affect the occurrence of *M. robertsii* in an organic cropping system**

Mary Barbercheck, Puneet K. Randhawa, Christina Mullen Department of Entomology, Penn State University, University Park, PA 16802, USA

8.30 **133** **Transmission of *Beauveria bassiana* and *Metarhizium anisopliae* to male *Bactrocera tryoni* via para-pheromone lures and subsequent transmission to females**
Ian R. Newton, Stefano De Faveri Department of Agriculture and Fisheries, Queensland, Australia

8.45 **134** **Efficacy of entomopathogenic fungi and *Bacillus thuringiensis* isolates against the invasive Fall Armyworm, *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae)**
Akutse K. S., Kimemia J.W., Ekesi S., Khamis F.M., Ombura O.L., and Subramanian S., International Centre of Insect Physiology and Ecology (*icipe*), P.O. Box 30772-00100, Nairobi Kenya

9.00 **135** **Susceptibility of *Spoladea recurvalis* (Lepidoptera: Crambidae) to entomopathogenic fungal and *Bacillus thuringiesis* (*Bt*)- based biopesticides**
Selpha Opisa[1,2*], Hannalene du Plessis[2], Komivi Senyo Akutse[1], Komi Kouma Mokpokpo Fiaboe[1] and Sunday Ekesi[11]International Centre of Insect Physiology and Ecology (icipe) P.O. Box 30772-00100, Nairobi, Kenya, [2] Unit for Environmental Sciences and Management, North-West University, Private Bag X6001, Potchefstroom 2520, South Africa

9.15 **136** **Impact of geographic location on mosquito microbiota**
Ephantus J. Muturi[1], Christopher Dunlap[1], Jose L. Ramirez[1], Alejandro P. Rooney[1], Chang-Hyun Kim[2] [1]Crop Bioprotection Research Unit, Agricultural Research Service, U.S. Department of Agriculture, 1815 N. University St. Peoria, IL. 61604. [2]Illinois Natural History Survey, University of Illinois at Urbana-Champaign, 1816 S. Oak St., Champaign IL 61820.

9.30 **137** **A plant-derived protein with insecticidal activity against Western Corn Rootworm**
Mark E. Nelson[1], Claudia Pérez Ortega[1], Jennifer Barry[1], Lu Liu[2], Gusui Wu[2] and Rodrigo Sarria [3]Corteva Agriscience™, Agriculture Division of DowDuPont, [1]Johnston, IA, U.S.A; [2]Hayward, CA, U.S.A; [3]Indianapolis, IN, U.S.A

9.45 **138** Cancelled

10-10.30 MORNING TEA

10.30-12.30 Pipeline
SIP General Membership meeting

12.30-13.30 LUNCH (NOT supplied)

Cross Divisional Symposium (Virus & Diseases of Beneficial Insects)

Thursday 13.30-15.30 Pipeline

White Spot Syndrome Virus – Emergence and control

Organisers/Moderators: Kelly Bateman and Just Vlak

13.30 **139-STU** **Overview of WSSV and its emergence**
Jie Huang, Xuan Dong, Xiaoyuan Wan, Yan Liang, Bing Yang, Qinghui Liu, Xiaoling Song, Xiuhua Wang, Qingli Zhang, Chengyin Shi. Laboratory for Marine Fisheries Science and Food Production Processes, Qingdao National Laboratory for Marine Science and Technology; Key Laboratory of Maricultural Organism Disease Control, Ministry of Agriculture and Rural Affairs; Qingdao Key Laboratory of Mariculture Epidemiology and Biosecurity; Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences; Qingdao 266071, China

14.00 **140** **White spot disease outbreak in farmed prawns in Queensland, Australia in 2016**
Peter Mohr[1], Nicholas Moody[1], Mark Crane[1], Debbie Eagles[1] Stephen Wesche[2], Kerrod Beattie[2], Allison Crook[21]CSIRO Australian Animal Health Laboratory, Geelong, Victoria, Australia; [2]Biosecurity Queensland, Queensland Department of Agriculture and Fisheries, Brisbane, Queensland, Australia

14.30 **141** **Wild type relative of the most important viral pathogen in global aquaculture**
K.S. Bateman[1,2]**,** J. Bojko2, J. Vlak[3], R. Kerr[1,2], K.F. Clark[4,5], S.E. Stewart-Clark[5], P. Byrne[6], S.J. Greenwood[4], D. Bass[1], G.D. Stentiford[1,2], R. van Aerle[1,2].[1]European Union Reference Laboratory for Crustacean Diseases, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Barrack Road, The Nothe, Weymouth, Dorset, DT4 8UB, UK. [2]Centre for Environment, Fisheries and Aquaculture Science (Cefas), Barrack Road, The Nothe, Weymouth, Dorset, DT4 8UB, UK. [3] Department of Plant Sciences, Laboratory of Virology, Wageningen University, Droevendaalsesteeg 1, 6708PB Wageningen, Netherlands. [4]Dept. of Biomedical Sciences and AVC Lobster Science Centre, Atlantic Veterinary College, University of Prince Edward Island, 550 University Avenue, Charlottetown, PEI, C1A 4P3, Canada. [5]Department of Plant and Animal Sciences, Agricultural Campus, Dalhousie University, PO Box 550, Truro, NS, B2N 5E3, Canada. [6]Department of Fisheries and Oceans Canada, Charlottetown, PEI, Canada.

15.00 **142** **Potential future therapies for WSSV**
Ornchuma Itsathitphaisarn[1,2], Siripong Thitamadee[1,3], Timothy W. Flegel[1,4]**,** Kallaya

Sritunyalucksana[5] [1]Center of Excellence for Shrimp Molecular Biology and Biotechnology (Centex Shrimp), Faculty of Science, Mahidol University, Bangkok, Thailand[2]Department of Biochemistry, Faculty of Science, Mahidol University, Bangkok, Thailand. [3]Department of Biotechnology, Faculty of Science, Mahidol University, Bangkok, Thailand. [4]National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Pathumthani, Thailand. [5]Shrimp–Pathogen Interaction Laboratory (SPI), National Center for Genetic Engineering and Biotechnology (BIOTEC), Bangkok, Thailand

Contributed papers Maui 3

Thursday 13.30-15.30

Nematodes 1

Moderators: Patricia Stock and Ivan Hiltpold

13.30 **143** **Effect of Temperature on the Infectivity of different Entomopathogenic Nematodes (Steinernematidae and Heterorhabditidae) Isolated from Natural Ecosystems**
Yara El Khoury[1,2], Monica Oreste[2], Elise Noujeim[1], Nabil Nemer[3], **Eustachio Tarasco**[2] [1]National Center for Marine Sciences, National Council for Scientific Research -CNRS, P.O.Box 11-8281, Ryad El Solh 1107 2260, 59, Zahia Selman Street, Beirut, Lebanon; [2]Department of Soil, Plant and Food Sciences, Section of Entomology and Zoology, University of Bari 'A. Moro, Bari, Italy; [3]Holy Spirit University of Kaslik, Faculty of Agricultural and Food Sciences, PO Box 446, Jounieh, Lebanon

13.45 **144** **Natural occurrence of entomopathogenic nematodes (*Rhabditida: Steinernematidae* and *Heterorhabditidae*) in areas infested by *Popillia japonica* (*Coleoptera, Scarabaeidae*) in Northern Italy**
Giulia Torrini[1], Francesco Paoli[1], Leonardo Marianelli[1], Stefania Simoncini[1], Claudia Benvenuti[1], Gian Paolo Barzanti[1], Giuseppe Mazza[1], Giovanni Bosio[2], Davide Venanzio[2], Emanuela Giacometto[2], **Eustachio Tarasco**[3], Giuseppino Sabbatini Peverieri[1], Pio Federico Roversi[1] [1]CREA Research Centre for Plant Protection and Certification, Firenze, via di Lanciola 12/a, Italy; [2]Settore Fitosanitario e Servizi Tecnico-scientifici, Regione Piemonte, via Livorno 60, Turin, Italy; [3]Department of Soil, Plant and Food Sciences, Section of Entomology and Zoology, University of Bari 'A. Moro, Bari, Italy

14.00 **145** Cancelled

14.15 **146** **Photo-biology: a tool to identify nematodes**
Ivan Hiltpold[1], Aron J. Owens[2], Anthony Ragone[2] [1]Departement of Entomology and Wildlife Ecology, College of Agriculture, University of Delaware, Newark, DE, USA; [2]Speckciton Bioscience LLC, Wilmington, DE, USA

14.30 **147** **Symbiont-mediated thermal tolerance in *Steinernema puntauvense* (Honduras strain) nematodes**
Danielle Noumeh[1], Brittany F. Peterson[1], S. **Patricia Stock**[1,2,3,] [1]Center for Insect Science, University of Arizona, Tucson, AZ. 85721; [2]Department of Entomology, [3]School of Animal and Comparative Biomedical Sciences

14.45 **148** **Mermithid parasitism of shoot borer (*Conogethes punctiferalis*) infesting ginger and turmeric and its biocontrol potential**
Senthil Kumar C.M.[1], Jacob T.K.[1], Devasahayam S.[1], Hariharan V.[1], Sharon D'Silva [1 1] ICAR - Indian Institute of Spices Research, Marikunnu P.O., Kozhikode, Kerala

15.00 **149** **Management of *Halyomorpha halys* by entomopathogenic nematodes in Georgia**
Nona Mikaia, Sokhumi State University, Department of Natural Sciences and Health Care, Tbilisi, Georgia

Contributed papers Maui 1 & 2

Thursday 13.30-15.30

Bacteria 3

Moderator: Marianne P. Carey

13.30 **150** ***Aedes* cadherin is an essential gene targeted by Cry11A**
Jianwu Chen, Karlygash Aimanova and **Sarjeet S Gill** University of California, USA

13.45 **151** **Role of *Bacillus thuringiensis* Cry1A toxins domains in the binding to the ABCC2 receptor from *Spodoptera exigua***
María Martínez-Solís[1], Daniel Pinos[1], Leivi Portugal[2], Juan Ferré[1], Salvador Herrero[1], **Patricia Hernández-Martínez** [11]ERI de Biotecnología y Biomedicina (BIOTECMED), Department of Genetics, Universitat de València, 46100 Burjassot, Spain. [2]Instituto de Biotecnología, Universidad Nacional Autónoma de México. Apdo. Postal 510-3, Cuernavaca 62250, Morelos, Mexico

14.00 **152** **Scale-free genetic interaction networks in *Heliothis virescens* challenged with *Bacillus thuringiensis* toxin Cry1Ac**
Ashoka D. Polpitiya[1], Jerreme Jackson[2], Cris Oppert[2], Juan Luis Jurat-Fuentes[2], and **O. P. Perera**[3]. [1]Office of Research and Innovation Services, Sri Lanka Technological Campus,

Padukka, Sri Lanka; [2] Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN, USA; [1]Southern Insect Management Research Unit, USDA-ARS, Stoneville, MS, USA

14.15 **153** **Transcription of the cellobiose transport pathway is controlled by Sigma 54 and regulated by CelR in *Bacillus thuringiensis***
Qi Peng, Haijian Cheng, Jie Zhang, Fuping *Song,* State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China

14.30 **154** **Polycalin is involved in the action mode of Cry2Aa toxin and resistance mechanism of Cry1Ac toxin in *Helicoverpa armigera* (Hübner)**
Bingjie Wang[1, 2], Yanan Wang[1], Jizhen Wei[1], Chen Liu[1], Lin Chen[1], Myint Myint Khaing[1], **Gemei Liang[1].** State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, P. R. China; 2. Environment and Plant Protection Institute, Chinese Academy of Tropical Agricultural Sciences

14.45 **155** Cancelled

15.00 **156** **Genome-wide analysis of ATP-binding cassette (ABC) transporters in the cotton bollworm, *Helicoverpa armigera***
Yutao Xiao Agricultural Genomes Institute at Shenzhen, Chinese Academy of Agricultural Sciences (AGIS/CAAS)

19.00 ---CONFERENCE BANQUET

Abstracts

Plenary Symposium –

Monday 10.30-12.30 Pipeline

Insect Pathology and Microbial Control – progress and prospects in the Asia-Pacific region

Organisers/Moderators: Trevor Jackson and Caroline Hauxwell

PLENARY SESSION MONDAY 10.30 **1**

Microbial control in New Zealand

Travis R. Glare[1], Maureen O'Callaghan[2]

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New Zealand is dependent on agriculture and forestry exports for economic well-being, so control of invertebrate pests is very important. There is a reasonably long history of microbial control attempts in New Zealand since European colonisation around 200 years ago. Beauveria was imported as a potential control agent of codling moth in the 1880s. Since then many invertebrate pathologists have been active, from the mycologists and virologists in the 1970's-80s, to the bacteriologists in the 1990s on. Currently there are around 17 microbial-based insecticides registered in New Zealand. Several more are currently under development including some novel disease causing agents. The development of several indigenous bacterial strains with novel combinations of toxins as commercial biopesticides is underway, as well as new endophytes for use in crop protection. In this talk the current state of microbial control use and commercialisation in New Zealand will be discussed, including the regulatory environment and industry approaches.

PLENARY SESSION MONDAY 10.50 **2**

Microbial control in pest management and IRMS in Australia

Caroline Hauxwell

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Australia's use of microbial control has developed from pioneering research to widescale adoption by mainstream agriculture and commercial manufacturing. Successes include the development and use of *Metarhizium acridium* for control of plague locusts, and the adoption and production of Baculoviruses for control of lepidopteran pests across a range of crops. Their commercial success has been both driven by and, in turn, supported the integration of microbial biopesticides into insecticide resistance management strategies (IRMS) and 'area wide' management programs, particularly in broadacre cropping.

The alternative 'mode of action' of insect pathogens, the maintenance of beneficial insects, and the interaction with evolution of resistance to chemical insecticides have underpinned their use in sophisticated industry practices for IRMS, and the Australian cotton and grains industries have been at the forefront of research developing formulation, application and agronomic practices that have supported efficacy and adoption. Australia's vast and largely unexplored microbiology, it's proximity and similarity in environment and pest species to Asia, and advanced research and legislative frameworks suggest that Australia has much more to offer in the future. This presentation will explore the development of microbial control in IRMS in Australia, and discuss some of the challenges in legislation, registration and research that may constrain or support it.

PLENARY SESSION MONDAY 11.15 **3**

Progress of *Bacillus thuringiensis* research and application in China

Ming Sun

State Key Laboratory of Agricultural Microbiology, College of Life Science and Technology, Huazhong Agricultural University, Wuhan, Hubei 430070, China

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China has made great advances in research and application of *Bacillus thuringiensis* (Bt) in recent decades. Bt insecticides and Bt transgenic cotton are widely used to control pests in the field, and some recombinant Bt products and transgenic Bt rice and corn have passed safety assessment protocols and have been awarded safety certification for GMOs. China has a good tradition of working to exploit Bt resources by collecting and identifying new strains, novel toxin genes, identifying new activities with expanded host spectrum and finding new active components. Since the first Class I holotype crystal protein gene (cry51Aa1) was identified in 2006, twenty Class I type genes have been isolated and many novel genes are awaiting nomenclature from the Bt Toxin Nomenclature Committee. Research is also focussing on the interaction of Bt with its host and/or environment, the evolution of Bt populations and pathogenic factors, as well as the development of new Bt insecticides and transgenic plants. Further details will be discussed in the presentation.

PLENARY SESSION MONDAY 11.40 **4**

Microbial control as a component of IPM in the prod**uction of oil palm in Malaysia**

Norman Kamarudin, Mohd Mazmira, Mohd Masri and Idris Abu Seman

Malaysian Palm Oil Board, No 6, Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia

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The bagworms (*Metisa plana* and *Pteroma pendula*) (Lepidoptera: Psychidae) have been attacking oil palm plantations particularly in the northern and southern parts of Malaysia. Integrated Pest Management (IPM) of bagworms is being implemented by aerial spraying of *Bacillus thuringiensis* (Bt), cultivation of beneficial plants and fixing of natural pheromone traps. With correct timing and strategy, the aerial spraying operation has successfully reduced the bagworm population below the economic threshold. The aerial spray of Bt also has no negative effects to the bagworm's natural enemies, in the presence of beneficial plants, hence providing long-term control of the bagworm. The pheromone trap is applied in the interim of bagworm generations to trap the male adults which reduces the chances of mating for the succeeding generation. The rhinoceros beetle, *Oryctes rhinoceros* (Coleoptera: Scarabaeidae) is a notorious pest of immature palms especially during replanting. The approach of IPM for rhinoceros beetle includes the use of *Metarhizium*, for control of the pest in its breeding sites and the Oryctes nudivirus (OrNV) for propagation of virus within the colonies. Different types of OrNV showed varied effectiveness on the different life stages of rhinoceros beetles and have high potential for controlling the pest. The subterranean termite, *Coptotermes curvignathus* (Isoptera: Rhinotermitidae) is a major insect pest for oil palm planted on peat. The termite infestation can be found on oil palm as early as 12 month after planting and could kill palm up to 5.3%. Two entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae* have shown potential as biological control agents. Both *M. anisopliae* and *B. bassiana* gave more than 60% control, similar to the use of insecticide (Fipronil). For the control of the devastating basal stem rot (BSR) disease or Ganoderma, the Integrated Ganoderma Management (IGM) approach was implemented, which includes sanitation, biological, fertilizer with beneficial (trace) elements and chemical control. These control measures are aimed at minimising disease incidence in replanting, prolonging the productive life of the infected palm, and delaying the progress of Ganoderma infection. Some BSR disease control methods in existing plantings and management strategies at replanting have been achieved and are being implemented in several oil palm plantations and smallholders in Malaysia. Some of the challenges which need to be addressed include the implementation of proper census and detection methods, awareness of suitable and effective chemicals for long term control, environmental manipulation to reduce pest outbreaks and enhanced surveillance of pests and diseases among smallholders. Increased efforts in these areas would ensure better management of pests and disease management for a sustainable and long term control of pest and diseases in oil palm.

PLENARY SESSION MONDAY 12.05 **5**

Microbial control for the Pacific Island states

Sean D.G. Marshall[1] and Trevor A. Jackson[1]

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The Pacific Islands (encompassing the islands of Melanesia, Polynesia and Micronesia) comprise about 30,000 islands in the Pacific Ocean, with a land mass of approximately 550,000 km^2 of land spread across 180 million km^2 of ocean (~36% of the earth's surface). Of the approximately 2000 inhabited islands, many are less than 10 km^2 (many atolls are less than 1 km^2). The terrestrial, freshwater and marine ecosystems of the Pacific Islands support more rare, endangered and threatened species than anywhere else on earth, and they also provide important natural resources necessary for the economies and cultures of many island communities. However, the Pacific Region is also experiencing rapid economic growth and a rising human population, which is accompanied by fragmentation and destruction of habitats associated with climate change, coastal development, pollution, agricultural expansion, and invasive species. Clearly balancing national priorities with sustainable practices and environmental protection in the use of these resources poses challenges. Within the Pacific Islands there is a significant reliance on availability of clean freshwater, subsistence agriculture, and the commercial farming sectors for health, food security, and the economy. Additionally, the rate of invasive invertebrate species introductions are rapidly increasing, and severely impacting economies, freedom to trade, sustainable development, health, ecosystem services, and resilience to natural disasters. Increasingly, primary producers and conservation efforts are under pressure to use alternatives to chemicals for pest control as there are concerns over human health, environmental impacts, and the emergence of pesticide resistance. The use of entomopathogens provides an alternative to synthetic pesticidal strategies for invertebrate pest management, but the level of use remains modest and inconsistent (though more recently has been rapidly gaining widespread support). Here we examine the historical and current use of invertebrate pathogens as microbial biocontrol agents of invertebrate pests in the Pacific Islands and will discuss in relation to future opportunities.

Symposium 1 (Nematodes)

Monday 13.30-15.30 Pipeline

Use of parasitic Nematodes to Control Pine-killing Woodwasps

Organisers/Moderators: Ann Hajek and Helen Nahrung

Symposium Monday 13.30 **6**

Control of sirex using the nematode *Beddingia siricidicola*: Past, Present and Future

Robin Bedding

CSIRO, Australia

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The European woodwasp, *Sirex noctilio*, killed millions of pines in New Zealand, Australia, South America and South Africa. Recently arrived in North America it poses a threat to 58 million hectares of pine forests in Southern USA. Collections from 29 countries, 31 tree species and 2 fungi yielded 7 species of *Beddingia* from 19 siricid species and 12 insect parasitoids but, only four strains of one species of one species of nematode, *B. siricidicol*a, were suitable for release. One form of *B. siricidicola* breeds within and sterilises female sirex while another, very different form feeds and breeds on the sirex's symbiotic fungus as the latter grows within the tree. Use of the free-living cycle, which can continue indefinitely, enabled establishment and storage of hundreds of monoxenic cultures for later evaluation and a means of mass rearing suitable nematodes for liberation. Nematodes properly inoculated into sirex killed trees breed throughout the tree, change form near sirex larvae and result in emerging parasitized sirex ovipositing nematode filled eggs into other trees often attacked by multiple sirex. At high levels of sirex infestion nematode parasitism approaches 100% followed by population collapse. However, control problems have arisen. In Australia, years of continual fungal culture resulted in loss of infectivity to sirex; this was resolved by re-isolating and liquid nitrogen freezing the favoured strain, liberated in 1970s, and removing and re-culturing it annually for liberation. In South Africa, trees dry before the nematodes can fully migrate but possibly complete adaptation of nematode strain to feeding on SA sirex symbiont might provide an earlier start to nematode breeding and migration. In North America where nematodes do not enter sirex eggs, release of juveniles from parent nematodes before sirex egg hardening might be achieved using the New Zealand strain.

Symposium Monday 14.10 **7**

Mechanisms responsible for *Sirex noctilio* nematode biocontrol program disruption in Australia

Fazila Yosuf[1], **Angus Carnegie**[2], Robin Bedding[3], Dick Bashford[4], Catherine Clarke[1], Geoff Gurr[1]

[1]Charles Sturt University, NSW; 2NSW Department of Primary Industries – Forestry, Sydney, NSW; 3CSIRO, Canberra, ACT; NSW Department of Primary Industries – Forestry, Sydney, NSW; Hobart, Tasmania.

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Sirex noctilio F., is an invasive wood wasp pest that can cause significant tree mortality to *Pinus* plantations in its introduced range (Southern Hemisphere, northeastern North America and northeastern China). A parasitic nematode, *Beddingia siricidicola* (Bedding), has been used to effectively manage *S. noctilio* populations. The nematodes feed and reproduce on the symbiotic fungus of *S. noctilio*, *Amylostereum areolatum* (Chaillet ex Fr.) Boidin and parasitises the *S. noctilio* larvae. Nematode parasitism does not kill *S. noctilio* but sterilises the females, which lay 'packets' of nematodes rather than eggs. This effect is important in the spatial and temporal distribution of the agent in the host population. In recent years, this historically successful biocontrol program has been disrupted in Australia by an invasive bark beetle, *Ips grandicollis* Eichhoff. Several field and laboratory studies have been conducted to understand the mechanisms that could be responsible for such disruption. Interactions between the invasive pest species, especially those mediated by microbial associates and their effect on the nematodes, are explored. The impact of elevated temperatures on complex insect-microbe interactions is also investigated. Results show that *A. areolatum* is a slower growing fungus than *Ophiostoma ips* (Rumbold) Nannfelt (beetle associated fungus) and is unable to grow in areas occupied by *O. ips. Ophiostoma ips* grows more rapidly at elevated temperature than *A. areolatum*. Furthermore, *I. grandicollis* infestation accelerates the wood drying, affecting the growth of *A. areolatum*, which consequently influences the survival and growth of *B. siricidicola*. The nematodes prefer to feed and lay eggs on *A. areolatum* than *O. ips*, but the presence of *O. ips* negatively affect the choice response of and the number of eggs laid by the nematodes. Temperature also plays a significant role in the growth and development of *S. noctilio.* The emerging females are bigger in size with maximum nematode- sterilised eggs at 24°C than at higher temperatures. Modest rises in temperature reflect climate change scenarios, which result in smaller sized *S. noctilio* with lowered nematode-sterilisation rate in females. Studies show that the competitive interactions between *O. ips* and *A. areolatum* are demonstrated to be key factors in the negative effect of *I. grandicollis* on *S. noctilio* biocontrol program, which may be exacerbated under climate change conditions.

Symposium Monday 14.30 **8**

Predicting *Sirex* biocontrol success in subtropical Australia: can *Deladenus siricidicola* take the heat?

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The invasive exotic woodwasp *Sirex noctilio* (Hymenoptera: Siricidae) established in the temperate pine plantation estate (*Pinus radiata* and *P. taeda*) in southern Queensland in 2009, fifty years after first reaching Australia. If it spreads further northwards, the wasp will encounter warmer climatic conditions and completely different host taxa - synthetic hybrids between *P. elliotti* and *P. caribaea* – than in its current range. However, subtropical conditions and these new potential host trees may impact substantially on the efficacy of existing successful biological control, in particular the major biological control agent, the nematode *Deladenus* (=*Beddingia*) *siricidicola*. We tested the performance of this important bicyclic nematode under subtropical conditions and in hybrid pine hosts. We also used probes to measure internal tree temperatures in the field in healthy and stressed trees to ascertain the conditions to which nematodes would be exposed in subtropical climate and hosts, and used these data to further predict nematode survival. Our results suggested that *D. siricidicola* may not be as effective in hybrid pine taxa as it is in current host taxa, possibly because of reduced growth of its mycetophagous-phase food source, *Amylostereum areolatum*, in hybrid pine. If *Sirex* reaches the coastal subtropical hybrid plantation estate, new nematode strains and/or alternative biological control agents may be required.

Symposium Monday 14.50 **STU-9**

Genetic diversity in global collection of *Deladenus siricidicola*

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The nematode *Deladenus siricidicola* is used as a biological control agent against the woodwasp *Sirex noctilio* that has invaded various regions of the world and that infests *Pinus* trees. The nematode was discovered parasitizing *S. noctilio* in New Zealand in the 1960s. Collections were subsequently made in Asia, Europe and North America to select a virulent and effective strain to use in biological control programs. This selection and the selection of other strains in Australia, South America and South Africa could have led to a narrow genetic diversity in nematode populations available for biological control programs. There are limited studies that have characterized the population genetics of these nematodes, and none from native regions. In this study, the patterns of population genetic diversity of *D. siricidicola* was characterized in collections from 10 countries, representing the native range (Spain), areas of accidental introduction (Canada, New Zealand and USA) and countries of intentional release (Argentina, Australia, Brazil, Chile, and South Africa). Characterization was achieved using mitochondrial (cytochrome oxidase subunit 1, COI) and nuclear (12 microsatellite markers) DNA diversity data. The results revealed the presence of four distinct lineages, i.e. Lineage A (from Canada, New Zealand and USA), Lineage B (from Argentina, Australia, Brazil, New Zealand and South Africa), Lineage C (from Spain and Australia) and Lineage D (from Australia). Lineage A was dominant in North America and appears to have originated from Europe, but is clearly distinct from the Lineage C found in Spain. Lineage A appears to have been introduced into Chile from North America, where it has interbred with populations of Lineage B. The dominant Lineage B in the Southern Hemisphere can be explained by the spread of the biological control programs originating from a selected strain, commonly referred to as the Kamona strain. Interestingly this strain also occurs in New Zealand, although it was never intentionally released in that country. Lineage D was found only in Australia and its origin is unknown. Overall, this study revealed considerable genetic diversity in *D. siricidicola* on a global scale and this provides an important resource for future use in biological control programs.

Symposium Monday 15.10 **10**

Potential for Non-target effects using biological control nematode against *Sirex noctilio* in North America

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In 2004, invasive *Sirex noctilio*, which is native to Eurasia, was first found in northeastern North America, where Sirex species and associated biotic communities are also native. *Sirex noctilio* had been introduced to numerous countries in the Southern Hemisphere and a strain of the nematode *Deladenus siricidicola* that sterilizes females had been used extensively for control. By 2009, we realized that a strain of *Deladenus siricidicola* that did not sterilize females was already present in *S. noctilio* populations in North America. When the non-sterilizing *D. siricidicola* infect, adult *S. noctilio* are smaller, and therefore non-sterile but infected females lay fewer eggs. Questions were raised regarding whether the commercialized sterilizing strain of *D. siricidicola* (Kamona) should be released for biological control or if this would impact native nontargets (which are not pests) significantly. We learned that non-sterilizing *D. siricidicola* is horizontally transmitted to the native pine-dwelling *Sirex nigricornis* that co-occurs in trees with *S. noctilio*. *Deladenus siricidicola* has also rarely been found within an associated wood boring beetle. When testing Kamona against *S. noctilio*, we found some hybridization between nematode strains (sterilizing x non-sterilizing), resulting in an intermediate level of sterilization. Whether Kamona will be introduced in North America for biological control of *S. noctilio* could also be determined by the efficacy of Kamona in controlling the strains of *S. noctliio* present in North America.

Contributed papers Maui 3

Monday 13.30-15.30

FUNGI 1

Moderator**:** Jarrod Leland

Contributed paper Monday 13.30 **11**

Global Trend in R&D of Biopesticides: 3Rs and e-Biopesticide

Jae Su Kim[1], Se Jin Lee[1], Jong Cheol Kim[1], Sihyeon Kim[1], Mi Rong Lee[1], So Eun Park[1], Dongwei Li[1], Tae Young Shin[1], Taek Su Shin[2], Tae Hoon Kim[2], Pan Jung Ha[2], Tae Hyun Park[2]

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The main goal of pesticide R&D is to control and reduce the population density of target insect pests below an economic threshold and increase crop quality as an integrated crop management. However, the continuous emergence of chemical-mediated residual toxicity, such as neonicotinoid inducing bee collapse and insect resistance have been enforced the regulation of synthetic pesticides (3Rs: residual, resistance and regulation). Synthetic pesticides with novel mode of actions could be developed to overcome insect resistance and reduce environmental toxicity, but at the same time, as an alternative biopesticides with more efficacious control activity could be developed by the advanced technology. Future decisions to select more realistic control options probably depend on the speed of technological development in two areas, chemical and biological pesticides. Now, a strategic collaboration between synthetic pesticides and biopesticides has been progressed, such as M&A in Bayer, Syngenta, BASF with small biopesticide companies to consider high quality agricultural products in a food chain. However, in near the future, biopesticides could be a major asset in pest management thanks to the faster technological development compared to chemical pesticides. So far biopesticides have been developed with the standpoint of environmental safety, but technology of effective control and economic downstream processing could increase the value of biopesticides. In addition, ecological biocontrol should be seriously considered for successful field applications. Lastly in near the future, digital agriculture-based environmental controlling is attractable in enhancing fungal biological performance. A concept of e-biopesticide (ecological, economic downstream, efficacious, environmentally safe, and environment-mediated) could be properly combined with digital agriculture and accelerate the use of biological control agents in the future farming.

Contributed paper Monday 13.45 **12**

Cancelled

Contributed paper Monday 14.00 **13**

Mosquito - entomopathogenic fungi molecular interactions that define the outcome of infection

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Vector borne diseases such as dengue, Zika and malaria are rapidly increasing in incidence and geographical range, representing major burdens to global health. Fungal entomopathogens offer potential alternative control methods in the fight against mosquitoes and other vectors of human pathogens. Infection of invertebrate hosts by fungal entomopathogens occurs on contact by direct penetration of their cuticle, proliferating inside the insect body and eventually leading to host death. As the fungal entomopathogen penetrates the mosquito cuticle and disseminates throughout the hemocoel, it faces potent immune responses mounted by the mosquito host. The level and timing of this response will determine the fate of the infection in the mosquito body. This talk will discuss the temporospatial molecular interactions between the mosquito *Aedes aegypti* and diverse fungal entomopathogens. Understanding of such molecular interactions are important in determining the effectiveness of a biocontrol agent and in the design of novel mosquito control approaches.

Contributed paper Monday 14.15 **14**

Effect of entomopathogenic fungi and different immunosupressors on Colorado potato beetle defense systems and ontogeny

Olga Yaroslavtseva[1], Vadim Kryukov[1], Oksana Tomilova[1], Olga Polenogova[1], Maksim Tyurin[1], Maria Ganina[2], Elena Chernyak[2], Olga Luzina[2], Nariman Salakhutdinov[2], Sergey Morozov[2], Viktor Glupov[1]
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The use of natural products to enhance the efficacy of microbial agents is an important topic in biocontrol of pest insects, including control of Colorado potato beetle. We studied the effect of different microbial and plant metabolites (*Bacillus thuringiensis* and *Streptomyces avrrmitilis* toxins, usnic acid derivates etc.) on the defense systems and development of mycoses of Colorado potato beetle larvae. We found the synergy between fungi (*Beauveria, Metarhizium*) and the metabolites caused by changes in cellular and humoral immune responses, as well as alterations in morphological and biochemical properties of integuments, which stipulated different adhesion and penetration rates. Moreover we showed that disturbance of larval development caused by the metabolites has pronounced impact on the immunity, integument properties, and susceptibility to fungi. Toxic effects of the metabolites had a stable synergy with entomopathogenic fungi in the laboratory and field experiments. The stressors combinations can be promising for the development of highly efficient products against Colorado potato beetle larvae. The study was supported by grant of Russian Federation President (MK-6456.2018.11), Russian Foundation for Basic Research (project № 16-54-53033) and Russian Science Foundation (grant № 15-14-10014).

Contributed paper Monday 14.30 **15**

Arf and Rab GTPases play important roles in conidiation, trap formation, stress resistance and virulence in the nematode-trapping fungus *Arthrobotrys oligospora*

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Small GTPases form a huge superfamily consisting of the Arf, Ras, Rho, Rab and Ran subfamilies, they are versatile temporal and spatial regulators of virtually all cellular processes including signal transduction, cytoskeleton dynamics and membrane trafficking. *Arthrobotrys oligospora* is a representative nematode-trapping fungus that can produce adhesive networks to capture nematodes. In this study, the roles of four Rab and Arf GTPases were characterized by gene knockout in the fungus *A. oligospora*. The disruption of gene AoRab-7A hindered the mycelial growth in different media, the conidiation of ΔAoRab-7A transformants was almost abolished, and the transcription of four sporulation-related genes (AbaA, FluG, Hyp1 and VosA) was down-regulated compared to the wild-type strain (WT). Furthermore, the tolerance of the ΔAoRab-7A mutants to SDS (sodium dodecyl sulfate) and H_2O_2 was also significantly reduced compared to the WT, and the transcription of several genes related to environmental resistance, such as genes for catalase and trehalose synthase, was down-regulated. Meanwhile, the extracellular proteolytic activity was decreased. Importantly, the ΔAoRab-7A mutants were unable to produce traps and capture nematodes. However, the disruption of gene AoRab-2 only affected the conidiation slightly but non-significantly, while other phenotypic traits were unaffected. Moreover, the gene AoRab-7A was also involved in the autophagy induced by nitrogen deprivation in *A. oligospora*. Similarly, compared with the WT, the growth of ΔAoArf-GAP mutants became slow and its colony was irregular, especially, the number of conidia was greatly

reduced and produced malformed spores. Moreover, the nematocidal activity of the ΔAoArf-GAP mutants was also significantly reduced. But the disruption of gene AoArl had no influence on the phenotypic traits compared to the WT. Our results revealed that the Rab and Arf GTPases are involved in the regulation of mycelial growth, conidiation, trap formation, stress resistance and pathogenicity in the nematode-trapping fungus *A. oligospora.*

Contributed paper Monday 14.45 **16-STU**

Biological solution for entomopathogenic fungi-mediated management of Japanese pine sawyer beetle, *Monochamus alternatus*

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Japanese pine sawyer, *Monochamus alternatu*s Hope (Coleoptera: Cerambycidae) is considered as a serious forest pest in pine trees. However the application of synthetic pesticides over a large forest area is not feasible nor environmentally sound. In this study, we collected entomopathogenic fungi from forest soil and assessed their virulence against the adults using several application methods in laboratory condition. Two *Metarhizium anisopliae* isolates showed high virulence against the adults. The adults showed 53-60% of mortality when sprayed at 1.0 × 10^7 conidia/ml. Mycosis on cadavers were observed on the adults 6 days after treatments. In semi-field conditions, an application of one M. anisopliae isolate showed a virulence of 60% against the adult. Movement and behavior of the adults in a pine tree was carefully monitored to construct an effective fungal application strategy and some of potential was obtained in the semi-field conditions. Consequently, we confirmed the possibility of the fungal isolates in controlling the beetles. In near the future, we will investigate several factors which is possibly related to the forest pest management using entomopathogenic fungi in field conditions, given the importance of fungal formulation and practical application methods. In ecological pest management, colonization of the applied entomopathogenic fungi on the pine trees is one of the aspects to be seriously monitored.

Contributed paper Monday 15.00 **17-STU**

Entomopathogenic fungal library to control longhorned tick, *Haemaphysalis longicornis*

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Longhorned tick, *Haemaphysalis longicornis* (Ixodida: Ixodidae) is one of the vectors of severe fever with thrombocytopenia syndrome virus (SFTSV) in human. The ticks occurs in most of grass fields, and the use of pyrethroid insecticides induced pest resistance and environmental residual toxicity. Particularly the use of chemicals near residential areas where persons live become a big issue, so much environmentally safe control agents needs to be considered. Here in this work, our interest was given to the selection of highly virulent entomopathogenic fungi against *H. longicornis.* A total of 101 fungal pathogens collected from mountainous areas were assayed by a dipping the nymph stage of ticks into a conidial suspension (1×10^7 conidia/ml). Interestingly among several species, *Metarhizium* species showed high virulence and mycosis were observed in 7-15 days. Most of the selected isolates produced a large amount of conidia in Italian millet, rice and millet with thermotolerance at 40-45°C. Based on these results, we selected a couple of isolates with high virulence against *H. longicornis* and they could be used for the control of the ticks after the set-up of a practical application strategy in fields by optimizing fungal colonization in soil and phyllosphere.

Contributed paper Monday 15.15 **18-STU**

Targeting adult click beetles with the entomopathogenic fungus *Metarhizium brunneum*: Is it effective and are there reproductive trade-offs?

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The use of fungal entomopathogens to manage insect populations is becoming increasingly common, particularly due to concerns about the environmental and human health risks of synthetic chemical pesticides. Insect pathogens are usually used like chemical insecticides and are applied onto high density pest populations with the expectation of immediate control. However, they may be more effective in longer term control strategies which aim to reduce pest population build-up. Key aspects of this type of approach include determining whether targeting adults produces the desired reduction in reproduction and whether the insects experience negative sublethal effects which further reduce population build-up. Insects may experience trade-offs between disease resistance and other necessary life functions, such as reproduction. These trade-offs will depend on the life stage targeted and may be especially pronounced in conditions of nutritional scarcity. We challenged the adult click beetle *Agriotes obscurus* with the fungal entomopathogen *Metarhizium brunneum* and

monitored their reproductive output under conditions of either continual feeding or starvation. We also compared the response of beetles at different times in the season. The timing of oviposition, as well as numbers and sizes of eggs laid, were recorded. As expected, females who were challenged with the pathogen died earlier than unchallenged insects, and fecundity was reduced both in females that had been challenged with high concentrations of *M. brunneum* and those that were subjected to starvation conditions. In addition, seasonality also had an impact, with beetles challenged later in the season showing changes to their egg laying strategy. These results suggest that the challenges of both fungal exposure and nutritional scarcity reduced resources available for reproduction, and that the effect of these challenges will differ depending on the age and reproductive potential of the individual.

Contributed papers Maui 1 & 2

Monday 13.30-15.30

Viruses 1

Moderators: Zhihong Hu and Robert Harrison

Contributed paper Monday 13.30 **19**

The development of genetically modified baculoviruses for improved control of the false codling moth, *Thaumatotibia leucotreta* in South Africa

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Baculovirus biopesticides form an important part of an integrated pest management (IPM) programme used to control agriculturally important insects in the South African citrus industry. One insect of considerable importance is the false codling moth (FCM), *Thaumatotibia leucotreta.* This pest can not only cause fruit damage but is also of phytosanitary concern to some of South Africa's export markets. The baculovirus, Cryptophlebia leucotreta granulovirus (CrleGV), has been formulated into commercial products which have been used effectively against this pest for well over a decade. More recently, Cryptophlebia peltastica nucleopolyhedrovirus (CrpeNPV), which infects FCM, was isolated and characterised. While the commercialisation of this novel virus for use alongside CrleGV is vitally important, additional efforts must be made to further improve their efficacy. A potential avenue for achieving improved virulence relies upon deletion of the egt gene from the viral genome. Several studies have investigated egt deletion mutants, with results often indicating improvements in the speed of kill. However, regulation of genetically modified (GM) baculoviruses in many countries has stifled development and commercialisation for use in the field. In contrast, South African regulation provides a framework by which GM baculoviruses can be established. This study aims to develop techniques required for the generation of GM baculoviruses, which may offer improved virulence against FCM in the field. Two GM CrpeNPV constructs have been designed and are under development using long range PCR amplification and Gibson assembly methods. These constructs will evaluate the effect that deleting the egt gene in CrpeNPV will have on the virus' speed of kill. Each GM CrpeNPV construct will be used to transfect a newly established embryonic cell line derived from FCM eggs, enabling the generation of GM occlusion bodies (OBs). GM OBs will be employed in biological assays against FCM neonates to determine whether any changes in speed of kill can be measured. The development of GM baculoviruses with improved virulence may lead to the commercialisation of novel more efficacious biopesticides for use in South Africa.

Contributed paper Monday 13.45 **20**

Establishment of Baculovirus-expressed VLPs-Induced Syncytial Formation Assay for Flavivirus Antiviral Screening

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The baculovirus-insect cell expression system has been widely used for heterologous protein expression. In this study, we established a new method for antiviral screening targeting to glycoprotein E of Flavivirus based on the baculovirus expression system. ZIKV is a mosquito-borne flavivirus. It has been reported that ZIKV infection was associated with microcephaly and serious neurological complications. As currently no specific vaccines and drugs are commercial available, ZIKV infection has posed great threat to the public health. ZIKV E protein either prME expressed in insect cells can form virus like particles (VLPs), which could induce membrane fusion between cells, showing that the E protein, which is responsible for receptor binding, attachment and virus fusion during cell entry, was proper folding and retained the fusogenic ability in VLPs. The syncytia was significantly reduced by the anti-E specific polyclonal antibodies (pAb) in a dose-dependent manner. Even the AMS, a thiol-conjugating reagent, were also shown to have

inhibitory effect on the E protein induced syncytia and inhibits ZIKV infection by blocking viral entry. And the phenomenon of syncytial formation induced by E protein in insect cells is common among flavivirus, including Japanese encephalitis virus (JEV), Dengue virus (DENV) and tick-borne encephalitis virus (TBEV). This inhibition effect on syncytial formation can be developed as a novel, safe and simple antiviral screening approach for inhibitory antibodies, peptides or small molecules targeting to E protein of ZIKV and other flaviviruses.

Contributed paper Monday 14.00 **21**

The baculovirus per os infectivity factor (PIF) complex and its conservation in other invertebrate large DNA viruses

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Baculovirus oral entry is dependent on per os infectivity factors (PIFs). So far, 10 PIFs have been identified and some of them form a complex. However, the exact composition of the complex and how it is formed remain to be investigated in detail. In this study, by blue native PAGE, mass spectrometry and Western analysis, the native PIF complex of AcMNPV is determined to be about 500 kDa in size and comprised of 9 PIFs, all PIFs except PIF5. Nuclear-cytoplasmic fractionation suggests that the PIF complex is assembled in the cytosol prior to transport into the nucleus. Analyses of the recombinant viruses with individual pifs deletion showed that PIF1, 2 and 3 form a core-PIF-complex. Biochemical experiments demonstrate that the full PIF complex is relative tolerant to proteolysis, while individual PIF proteins degrade fast in the absence of a PIF complex. PIF homologs of two other invertebrate large DNA viruses, white spot syndrome virus and *Microplitis mediator* bracovirus, also form a multi-protein complex. These results imply that the PIF complex is an evolutionarily conserved machinery to allow entry of such viruses into their host and may provide protection of individual PIFs to protease degradation in the midgut of the host.

Contributed paper Monday 14.15 **22-STU**

Baculovirus oral infectivity is mediated by a complex interplay between per os infectivity factors

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Baculoviruses orally infect caterpillars when they eat leaf material that is contaminated with viral occlusion bodies. After oral ingestion, the occlusion bodies dissolve in the alkaline midgut, releasing occlusion-derived viruses (ODVs) that infect midgut epithelium cells. The ODV-envelope contains nine different so-called per os infectivity factors (PIF-proteins), which are essential for ODV oral infectivity; and seven of these PIFs form a complex. This entry complex consists of a stable core formed by PIF1, PIF2, PIF3 and PIF4, and loosely associated PIFs P74, P95 and PIF6. To determine how oral infectivity correlates with complex formation, we analysed PIF1 and PIF2 C-terminal truncation mutants of Autographa californica multiple nucleopolyhedrovirus. Truncation of either PIF1 or PIF2 severely impaired ODV oral infectivity, while formation of the core-complex was unaffected. However, formation of the entry complex did not occur anymore as P74 and P95 had dissociated from the core. Formation of the entry complex also failed in absence of either P74 of PIF6, suggesting that loosely associated PIFs mediate each other's association with the core as well. These findings indicate that ODV oral infectivity relies on interactions between the core and loosely associated PIFs.

Contributed paper Monday 14.30 **23-STU**

Aedes anphevirus (AeAV): an insect-specific virus distributed worldwide in *Aedes aegypti* mosquitoes has complex interplays with Wolbachia and dengue virus infection in cells

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The diversity of insect specific viruses (ISVs) present in the yellow fever mosquito *Aedes aegypti* remains poorly understood. Understanding ISV diversity is important as ISVs have been demonstrated to modulate transmission of arboviruses such as dengue virus (DENV) and West Nile virus within the mosquito vector. In this study, we characterised Aedes anphevirus (AeAV), a negative-sense RNA virus from the order Mononegavirales. AeAV identified from Aedes cell lines were infectious to both *Ae. aegypti* and *Aedes albopictus* cells, but not to three mammalian cell lines. To understand the incidence and genetic diversity of AeAV, we assembled 17 coding-complete and two partial genomes of AeAV from available RNA-Seq data. Phylogenetic analysis of AeAV strains indicates that as the *Ae. aegypti* mosquito has expanded into the Americas and Asia-Pacific, AeAV has evolved into monophyletic African, American and Asia-Pacific lineages. AeAV also appears to transmit vertically and be present in numerous laboratory colonies, wild-caught mosquitoes and cell lines. The endosymbiotic bacterium *Wolbachia pipientis* restricts positive-sense RNA viruses in *Ae. aegypti.* Re-analysis of a small RNA library of *Ae. aegypti* cells co-infected with AeAV and *Wolbachia* produces an abundant RNAi response consistent with persistent virus replication. We found *Wolbachia* enhances replication of AeAV when compared to a tetracycline cleared cell line, and AeAV modestly reduces DENV replication in vitro. The results from our study improve understanding of the diversity and evolution of the virome of *Ae. aegypt*i and adds to previous evidence that shows *Wolbachia* does not restrict a range of negative strand RNA viruses.

Contributed paper Monday 14.45 **24**

Natural baculovirus coinfection in *Spodoptera ornithogall*i larvae; advances in the characterization of Spodoptera ornithogalli nucleopolyhedrovirus (SporMNPV) and granulovirus (SporGV)

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The yellow striped armyworm, *Spodoptera ornithogalli* (Guenée) (Lepidoptera: Noctuidae), is a polyphytophagous insect widely distributed in the Americas from Canada to Argentina. The larvae feed on various plants of agricultural importance such as alfalfa, tomato, tobacco and onion, among others. In particular, *S. ornithogalli* can be a serious pest on transgenic cotton and ornamental flower crops in Colombia. In order to explore bioinsecticide agents to control populations of this insect, natural infected larvae of this species were recovered from the field and viruses were morphologically and molecularly characterized. Electron micrographs revealed the presence of two types of Occlusion Bodies (OBs) with polyhedral and granular shape, respectively. The viruses were separated by different steps of ultracentrifugation in sucrose gradients and controlled infection on lab reared larvae of *Spodoptera ornithogalli*, and the genomes were isolated and used as PCR template for baculovirus classification using primers for polh/gran, lef8 and lef9 genes. The bioinformatics analysis of the sequences showed that both isolates were new species of Baculoviridae, named as *Spodoptera ornithogalli* multiple nucleopolyhedrovirus (SporMNPV) and *Spodoptera*

ornithogalli granulovirus (SporGV). Subsequently, strategies of whole genome sequencing were carried out using Illumina technology. SporMNPV is a group II alphabaculovirus related with SpliNPV-II and SporGV was found in the betabaculovirus group. Baculoviruses coinfections have been reported previously in some insects in the field and have inspired studies about novel strategies for developing biopesticides.

Contributed paper Monday 15.00 **25**

Baculovirus as an efficient vector for gene delivery into mosquitoes

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Efficient gene delivery technologies play an essential role in the gene functional analyses that are necessary for basic and applied researches. Mosquitoes are ubiquitous insects, responsible for transmitting many deadly arboviruses causing high numbers of human deaths every year. The lack of efficient and flexible gene delivery strategies in mosquitoes are among the major hurdles for the study of mosquito biology and mosquito-pathogen interactions. We found that Autographa californica multiple nucleopolyhedrovirus (AcMNPV), the type baculovirus species, can efficiently transduce mosquito cells without viral propagation, allowing high level gene expression upon inducement by suitable promoters without obvious negative effects on cell propagation and viability. AcMNPV transduces into several mosquito cell types, efficiently than in commonly used mammalian cell lines and classical plasmid DNA transfection approaches. Moreover, AcMNPV can transduce both larvae and adults of essentially all blood-sucking mosquito genera, resulting in bright fluorescence in insect bodies with little or no tissue barriers. Our experiments establish baculovirus as a convenient and powerful gene delivery vector in vitro and in vivo that will greatly benefit research into mosquito gene regulation, development and the study of mosquito-borne viruses.

Contributed paper Monday 15.15 **26**

The 38K-mediated specific dephosphorylation of the viral core protein P6.9 plays an important role in the nucleocapsid assembly of Autographa californica Multiple Nucleopolyhedrovirus

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Encapsidation of the viral genomes, leading to the assembly of the nucleocapsids to form infectious progeny virions, is a key step in many virus life cycles. Baculovirus nucleocapsid assembly is a complex process that involves many proteins. Our previous studies showed that the deletion of the core gene 38K (ac98) interrupted the nucleocapsid assembly by producing capsid sheaths devoid of viral genomes by an unknown mechanism. All homologs of 38K contain conserved motifs of the haloacid dehalogenase superfamily, which are involved in phosphoryl transfer. The requirements of these motifs for nucleocapsid assembly, confirmed in the present study, suggest that 38K may be a functioning haloacid dehalogenase. P6.9 is also encoded by a core gene (ac100) and is required for viral genome encapsidation. It has been reported that multiple phosphorylated species of P6.9 are present in virus-infected cells, while only an unphosphorylated species is detected in the budded virus. Therefore, whether 38K mediates the dephosphorylation of P6.9 was investigated. An additional phosphorylated species of P6.9 in 38K-deleted or -mutated virus-transfected cells was detected, and the dephosphorylated sites mediated by 38K were determined by mass spectrometry. To assess the effects of dephosphorylation of P6.9 mediated by 38K on virus replication, these sites were mutated to glutamic acids (phosphorylation-mimic mutant) or to alanines (phosphorylation-deficient mutant). Studies showed that the nucleocapsid assembly was interrupted in phosphorylation-mimic mutant virus-transfected cells. Taken together, our findings demonstrate that 38K mediates the dephosphorylation of specific sites at the C terminus of P6.9, which is essential for viral genome encapsidation.

Microbial Control Division Symposium

Monday 16.00-18.00 Pipeline

The challenge of CRB-G to palm production in the Pacific and prospects for microbial control

Organisers/Moderators: Sean Marshall and Trevor Jackson

Symposium Monday 16.00 **27**

Progress with control of a virus resistant coconut rhinoceros beetle

Sean D.G. Marshall[1], Aubrey Moore[2], Mark Ero[3], Crispus Fanai[4], Maclean Vaqalo[5], Trevor A. Jackson[1]
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Oryctes rhinoceros (Linnaeus 1758) (Coleoptera: Scarabaeidae: Dynastinae) (coconut rhinoceros beetle; CRB) is a major pest of coconut and oil palm. The discovery and release of *Oryctes rhinoceros* nudivirus (OrNV) in the 1960s, 1970s, and 1980s allowed for the successful management of populations in Pacific Island Countries and Territories. Augmentative release of OrNV continues to be an important mechanism for CRB management in both coconut and oil palm growing regions. For ~40 years after adoption of this biocontrol strategy, no new outbreaks of CRB were reported from uninfested palm growing islands in the Pacific ensuring continuity of palm based village economies. However, the situation has recently changed. For first time in ~40 years, CRB invasion into completely new areas was reported in the Pacific – Guam (2007); Port Moresby, Papua New Guinea (2009); Honolulu, Hawai'i (2013); Honiara, Solomon Islands (2015); and Rota, Northern Mariana Islands (2017). Molecular analyses determined that all of these outbreaks were caused by a previously unrecognized haplotype, designated as CRB-G. Common to all new outbreak areas is the high incidence of severe palm damage not seen since the introduction of OrNV. Moreover, Palau, which was originally invaded by CRB in the 1940s and subsequently brought under control by OrNV, has also reported (2010) severe palm damage; the presence of a mixed CRB population that includes CRB-G. PCR analysis shows that OrNV is generally present at high incidence in established populations of CRB, but is absent from the invasive CRB-G populations. CRB-G from Guam was not susceptible to OrNV infection by oral delivery of commonly released isolates, but injection of the virus did cause mortality. We will discuss current results in relation to efforts currently being employed to slow the spread of CRB-G both within and between islands, and to identify longer term control measures for use against CRB-G (including novel virus isolates and other agents).

Symposium Monday 16.15 **28**

Attempted microbial control of coconut rhinoceros beetle, *Oryctes rhinoceros*, Biotype G on Guam using *Oryctes rhinoceros* nudivirus and *Metarhizium majus*

Aubrey Moore[1], Sean D. G. Marshall[2], Roland Quitugua[1], Ian Iriarte[1]
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Coconut rhinoceros beetle (CRB) was first detected on Guam during 2007. Following a failed eradication program, we attempted to establish effective microbial control using *Oryctes rhinoceros* nudivirus (OrNV) imported from AgResearch New Zealand and green muscardine fungus (GMF), *Metarhizium majus*, imported from the Philippine Coconut Authority. OrNV was initially released by autodissemination but did not result in population suppression. Molecular analyses indicated that the Guam population is genetically distinct from other populations which had invaded Pacific Islands and laboratory bioassays indicated that this genotype is resistant to infection by OrNV isolates commonly used and released in the Pacific region. This novel biotype has been named CRB Biotype G (CRB-G) and it is spreading throughout the Pacific. GMF was released by autodissemination and incorporation of spores into CRB breeding sites. An extensive post-release survey indicated that field mortality from GMF was between 10% and 38%. Population suppression by GMF coupled with breeding site sanitation and other integrated pest management tactics did not prevent an uncontrolled island-wide CRB outbreak triggered by Typhoon Dolphin which passed over Guam in May 2015. Adults emerging from abundant CRB breeding sites generated by this typhoon were numerous enough to kill large numbers of mature coconuts, initiating a positive feedback cycle whereby adults emerging from dead palms kill even more palms. History indicates that the most feasible way to stop the current outbreak on Guam is to find and release an OrNV isolate which is pathogenic for CRB-G. We are currently locating populations of CRB-G biotype within its native range and looking for OrNV isolates from these populations which can be used for effective microbial control.

Symposium Monday 16.30 **29**

Biotype and diversity of *Oryctes rhinoceros* in Japan

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Oryctes rhinoceros is important palm pest. It was distributed in South Asia and South East Asia, but spread to the Pacific islands in 1908. Oryctes nudivirus (OrNV) initially isolated in Malaysia was used as a biological control agent and its release in the Pacific island countries since the 1960s was successful in reducing damage by the pest. However, since 2007, damage of *Oryctes* emerged again in islands including Guam. It was shown that a new biotype of the beetle (biotype G) has emerged in the newly invaded areas and this biotype was more resistant to OrNV than the previously invading biotype. Our research objectives are to find the factors associated with resistance of *O. rhinoceros* to OrNV, and beetles showing resistant and susceptible traits were needed for the comparison. To comply with Nagoya Protocol, we initially examined the domestic population of *O. rhinoceros* in Japan. This species was firstly recorded in Ishigaki islands in 1921, but severe damage was recognized in Okinawa main island in 1975, and Amami island in 1991. We collected *Oryctes* beetles in Amami, Ishigaki and Okinawa islands to elucidate their biotype and biological properties of the populations.

Symposium Monday 16.45 **30**

CRB damage and resistance assessment in the Palau Archipelago

Christopher Kitalong[1], Justin Omak Ramarui[1], Jason Ngiramengior[1], Balang Skey[1], Nelson Masang[1,2], Shizu Watanabe[2], Michael Melzer[2], **Madoka Nakai**[3], Joel Miles[1].
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The Coconut Rhinoceros Beetle (CRB), a.k.a. *Oryctes rhinoceros*, is an invasive insect that kills coconut trees and other palm species by feeding on the plant's crown. Two biotypes of the CRB are in Palau: CRB-S & CRB-G. Damage Assessment Surveys were conducted on coconut trees in several locations in all of the 16 states in Palau in 2016, 2017 and 2018. These surveys were made to determine the amount of damage done by the beetles. Specialized traps were used along with manual searches through debris to collect and determine distribution of CRB-G as well as incidence of Oryctes Nudivirus (OrNV) infection. The results of the Damage Assessment Surveys show slow recovery/reduced damage in coconut tree fronds. Furthermore, analysis of biotype and viral detection show a very high rate of infection of all CRB with the nudivirus (CRB: 92%; CRB-G: 83%). The reduced tree damage and high rate of infection of the beetles, as well as visual assessments of CRB samples gut damage, lead to the preliminary conclusion that the OrNV in Palau CRB is virulent. Further assessment is necessary but immediate focus of all parties should be made to identify and test virulence of OrNV in Palau in order to control CRB for the region.

Symposium Monday 17.00 **31**

Infectivity of Malaysian Oryctes nudivirus (OrNV) propagated in insect cell line DSIR-HA-1179 against the rhinoceros beetle, *Oryctes rhinoceros*

Nur Ain Farhah & Ros Saidon Khudri[1], Norman Kamarudin1, Sean Marshall[2] and Ramle Moslim[3]
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The Oryctes nudivirus (OrNV) is a classical biocontrol agent for the notorious oil palm pest, rhinoceros beetle, *Oryctes rhinoceros*. The replication of the Malaysian OrNV types A, B, and C in cell line DSIR-HA-1179 was studied. Successful replication of OrNV in the cell line was confirmed by the appearance of cytopathic effects (CPE), Polymerase Chain Reaction (PCR) analysis, and the reduction in viable cell density of the infected cells as compared to the healthy cells. The infectivity of in vitro produced OrNV types A, B, and C and virus extracted from infected beetle guts were then evaluated on *Oryctes rhinoceros* larvae and freshly emerged adults. Virus was presented to larvae via oral inoculation and substrate contamination and all types of OrNV prepared from either source caused L3 larval mortality and infectivity. The highest mortality was 100%, on larvae treated by the cell-propagated OrNV type A and the highest infectivity was 46.7%, recorded by wild-collected OrNV type A. Infectivity of larvae was lower using the food substrate contamination method. OrNV type C recorded the highest infection of 26.7%, followed by type B (20.0%) and type A (13.3%). However, in experiments with adult beetles, OrNV type B was more virulent than the other types of OrNV as it produced the highest infectivity (88%) as well as the shortest lethal time (LT_{50} of 33.47 days). As the infectivity by OrNV increased feeding activity of the infected beetles decreased markedly, from 12 days after treatment. The results indicated that the selection of isolates and inoculation techniques produced different impacts on the larvae and adult rhinoceros beetle. The results also showed that OrNV produced from cell culture is infective and

that a standard evaluation method is needed for mass production of virus in future.

Symposium Monday 17.15 **32**

Coconut Rhinoceros Beetle (CRB) control efforts in oil palm: Papua New Guinea (CRB-P) versus Solomon Islands (CRB-G)

Mark Ero and Luc Bonneau
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The common biotype (CRB-P) of the coconut rhinoceros beetle (*Oryctes rhinoceros* L.) was first detected in Papua New Guinea (PNG) around the Rabaul area (East New Britain Province) on the New Britain Island in 1942. It was suspected to have arrived on ships carrying war supplies during World War II. It then spread to the neighbouring New Ireland and West New Britain Provinces. It has also been confirmed from the National Capital District (NCD) and the Central and Morobe Provinces on the mainland. The incursion caused severe damage to coconut palms. The NudiVirus (OrNV) was eventually introduced into the country from Samoa as part of the control programme after it was introduced there from Malaysia. It was first introduced into New Ireland then redistributed to the other parts of the country from there. The introduction gradually brought the beetle population under check. The pest occasionally causes economic damage to young replant oil palms (1-3 years old) when population builds up by breeding in fallen palm trunks that readily provides conducive breeding substrates. An integrated approach using pheromone trapping, natural infection by NudiVirus and Metarhizium infection has been used for the management of any infestation. However, the incursion of the Guam biotype *O. rhinoceros* (CRB-G) which is resistant to the NudiVirus has posed a greater challenge for control on both coconut and oil palms. The incursion in the Solomon Islands in replant plantations on oil palms has posed a major challenge for the oil palm industry. This presentation provides an overview of the infestation levels between the two biotypes and the outlines the control programmes that have been applied to manage each biotype.

Symposium Monday 17.30 **33**

The status of Coconut Rhinoceros Beetle, *Oryctes rhinoceros* (L) Scarabaeidae: Dynastinae, in Solomon Islands

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Oryctes rhinoceros was first confirmed as present in Solomon Islands in January 2015. Based on initial delimiting surveys it was determined that it had likely been there for at least two generations before it was detected and that consequently eradication was not an option. Soon after, scientists at AgResearch, New Zealand, confirmed it was the 'G' (Guam) strain of the species that is not susceptible to the nudivirus that controls another strain elsewhere in the South Pacific. An emergency response plan was developed but due to inadequate financing implementation has been inconsistent and weak, consequently the pest has spread and is now reported on parts of seven islands. More recently some funding is becoming available and management programmes are being implemented including: sanitation programmes to destroy breeding sites (rotting palm logs, green manure, compost and chicken manure), internal quarantines on shipping and the use of entomopathogens. Work to identify strains of virus effective against CRB-G is reported elsewhere in this symposium. Some preliminary details of work to introduce the fungus *Metarhizium anisopliae* are reported here.

Symposium Monday 17.45 **34**

Challenge of Coconut rhinoceros beetle Guam biotype (CRB-G) to Pacific

Maclean Vaqalo[1], Visoni Timote[1], Fereti Atu[1], Sean Marshall[2], Trevor Jackson[2]
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The continuous unintentional introductions of coconut rhinoceros beetle (CRB) since 1909 in the Pacific Islands from Southeast Asia have continued to greatly impact the livelihood of many Pacific Islanders that depend on coconut and other palms for food, water and economic security. Two dominant strains of CRB, defined by the difference in DNA and damage behaviours, are recognised present in the region. The older strain, CRB-P, has a known virus (Oryctes nudivirus, OrNV) that has effectively kept it under control, while the newer strain CRB-G which was detected since 2007 is tolerant and resistant to the virus. In terms of behavioural differences, CRB-G is invasive spreading to six Pacific Island countries and territories within 10 year. CRB-P has not been spreading for more than 40 years, which perfectly demonstrated the effect of coincidence release of OrNV since the1960s. Also, CRB-G damage has been observed to be killing palms as it continues to spread, while CRB-P only have outbreaks where OrVN is absent or breeding habitats are left unattended and managed to control the beetle. The Pacific Community (SPC) with its regional mandate to safeguard the livelihood of Pacific island citizens, has seriously considered complaints by both the affected and as well as highly threatened nations, and therefore developed a holistic programme approach to address the underlining issues. First and foremost, SPC believes in better coordination of nations, stakeholders including donors and technical

institutions, scientists, government, communities, farmers, and private sectors. SPC is better placed to coordinate the CRB Regional Programme approach in the Pacific. Other areas covered in the programme include strengthening of biosecurity awareness and surveillance, advocacy on restricting spread and control of CRB through plantation sanitation and clean up campaigns, developing researches on effective bio-agents, traps, pheromones and insecticides.

Contributed papers Maui 1 & 2

Monday 16.00-18.00

BENEFICIAL INVERTEBRATES & MICROSPORIDIA 1

Moderator**: TBA**

Contributed paper Monday 16.00 **35-STU**

A possible new species of *Tubulinosema* (Microsporidia: Tubulinosematidae) affecting silkworms (*Bombyx mori*) in Brazil

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Silk production is an economically important activity in Brazil and worldwide. Its fabrication is made by the silkworm, *Bombyx mori*, and can be seriously affected by diseases caused by entomopathogens that attack this insect. Microsporidia possess historical interest in impairing the world's silk production. This work aimed *in vivo* and *in vitro* investigations, as well as to make a phylogenetic analysis of Microsporidia isolated from a diseased silkworm, collected in a sericulture farm in Brazil. In vivo tests consisted of characterizing the spore size, the internal and external symptoms of infected larvae and the pathogenicity against *B. mori*. The in vitro tests consisted of the spore germination properties. Phylogenetic analysis was based on the analysis and comparison of sequences of the Small Subunit (SSU) genes of rRNA for genus identification. Also, the arrangements of the rRNA genes (SSU, ITS and LSU) were analyzed for subsequent sequencing of the ITS region for species identification. From the sequencing of SSU rRNA, it was possible to identify three different organisms infecting the *B. mori* larva, belonging to the genera *Endoreticulatus*, *Nosema* and *Tubulinosema*. The analysis of the rRNA gene arrangements for these three genera corroborated this identification. Sequencing of ITS enabled the identification of one of the organisms as *Nosema bombycis* and another as a possible new species of *Tubulinosema*. After the inoculation of the mixed microsporidian isolates in *B. mori*, a prevalence in the development and multiplication of only *Tubulinosema* spp. was observed. Measurement of spore size and PCR using primers specific for the three genera confirmed this identification. The results from pathogenicity bioassays showed a low mortality in *B. mori* (less than 10%) and, thus demonstrated that *Tubulinosem*a spp. causes a chronic infection of low pathogenicity, but leads to a drastic reduction of silk production. This study identifies a possible new species of *Tubulinosema* infecting *B. mori* in Brazil, and the symptoms related to its infection. However, further studies are needed to characterize the life cycle in the host, its way of transmission and its ultrastructural morphology through transmission electron microscopy for the confirmation of *Tubulinosema* spp. as a new species.

Contributed paper Monday16.15 **36-STU**

Transcriptomic analysis of *Rozella allomycis* and spliceosomal diversity of early-diverging fungi

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Microsporidia genomes are among the most reduced eukaryotic genomes and with this reduction has come the loss of many, and in some cases all, of the spliceosomal introns. This loss of introns has been mirrored by a loss of spliceosomal components in microsporidia. As an example, *Pseudoloma neurophilia* has 13 of the approximately 90 spliceosomal proteins in Saccharomyces cerevisiae. We previously showed that the remaining 36 introns of *Encephalitozoon cuniculi* were spliced at low levels, likely as a result of the reduced spliceosome. However, there was one exception, a relatively long intron, spliced at higher levels, likely due to a hyperextended branchpoint motif that we predict increases splicing via enhanced pairing with the U2 snRNA. This motif is conserved in similar introns across intron-containing microsporidia, although we could not identify it in the Rozellids, the sister group to the microsporidia that have similar intracellular parasitic life histories. We examined transcriptomes of *Rozella allomycis* and found 379 differentially expressed genes during the infective stage. *R. allomycis* has a more robust spliceosome consisting of 57 spliceosomal components and has approximately 20,000 introns. However, these introns do not appear to have the same extended spliceosomal motifs as microsporidia. These data not only provide us with insights into the evolution of genome reduction and by extension, intron loss, but also increase our understanding of the evolution history of microsporidia.

Contributed paper Monday 16.30 **37**

Controlling the pandemic of the microsporidian *Enterocytozoon hepatopenaei* in shrimp aquaculture: from molecular understanding to practical solutions

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Hepatopancreatic microsporidiosis (HPM) caused by the microsporidian, *Enterocytozoon hepatopenaei* (EHP) is currently a serious pathogen causing growth retardation in cultured shrimp across the Australasian region. The lack of treatment for EHP combined with its transmission via environmentally resistant spores facilitates its spread. To control the spread of EHP, research in our laboratory is divided into two areas. The first focuses on molecular and biochemical investigations to characterize its mechanisms of pathogenesis. From our whole genome study, one virulence factor is a spore wall protein (SWP) gene that we have used as the basis of a new and more specific EHP diagnostic method. Further investigations are underway into the role of SWP and other virulence gene that might be vulnerable targets for EHP control. The second area of research focuses on identification more immediate, practical and urgently needed methods to control EHP. To this end, we have identified conditions for purification of living spores of EHP and for induction of their polar tube extrusion, an event that initiates EHP entry into the host cell cytoplasm. Thus, abortive extrusion inactivates spores, preventing infection. At the same time the living, purified spores can be used for threshold infection studies, for infection trials with potential carriers, for quantitative inactivation tests using various disinfectants and other reagents, etc. Under this umbrella, we have also developed a cohabitation laboratory challenge model to facilitate further research on possible methods to block horizontal transmission in shrimp ponds and to treat already-infected shrimp. Recently, we have biochemically characterized SWP1 from EHP. EhSWP1 is be found on both endospore and exospore layers of spore wall. The heparin-binding property of EhSWP1 led to a model that heparin binding may be one of the early steps in the infection process of EHP.

Contributed paper Monday 16.45 **38**

The influence of microsporidian pathogens from commercially available lady beetles on non-target insect predators

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More than 70 species of natural enemies are available for pest control, including the aphid predators, *Adalia bipunctata* L. (two-spotted lady beetle) and *Hippodamia convergens* Guérin-Méneville (convergent lady beetle). The microsporidian pathogens Nosema adaliae and *Tubulinosema hippodamiae* were originally described from *A. bipunctata* and *H. convergens*, respectively. Spores of both pathogens are transmitted through beetle eggs, providing an opportune vehicle for pathogen transmission. When beetles are used in combination with other insect predators for biological pest control, there is opportunity for the transmission of these microsporidia to other, non-target insect predators, particularly those that readily feed on beetle eggs or larvae. Both *N. adaliae* and *H. convergens* infect other non-target native and introduced lady beetles, causing predictable, sub-lethal effects on the life history of these insects. Transmission of these pathogens has also been examined in non-beetle insect predators, including the green lacewing (*Chrysoperla carnea* Stephens), the spined soldier bug (*Podisus maculiventris* Say), and the Chinese mantis (*Tenodera sinensis* Saussure). Transmission and effects of microsporidia vary in these non-beetle hosts, which may influence the success of these insect predators.

Contributed paper Monday 17.00 **39**

A new species of microsporidia from the mosquito *Uranotaenia lowii* is related to the Hazardia clade

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Uranotaenia (Diptera: Culicidae) is a mosquito genus with a worldwide distribution and comprised of approximately 120 species. In Florida (USA) there are two common species, *Ur. lowii* and *Ur. sapphirina* with adults feeding mainly on cold blooded animals. The larvae inhabit a wide range of aquatic habitats and can be found throughout the year. These species are not considered to be vectors of arboviruses to humans but West Nile virus has been isolated from field collected *Ur. sapphirina* in the USA. Only one species of microsporidia (*Amblyospora nataliae*) has been formally described from Ur. nataliae collected in Argentina. In 1969 a *Stempellia* sp. was reported from *Ur. sapphirina* in Louisiana but was never formally described and there are no subsequent reports. Recently, a microsporidium was found in larvae of *Ur. lowii* in Florida that had pyriform spores similar to the *Stempellia* type. The SSU rRNA gene was sequenced and preliminary blast analysis found the closest match (93% identity) to be *Hazardia milleri* from the mosquito *Culex quinquefasciatus*. The next best matches (87% identity) were for *Lanatospora costata* isolated from a copepod and *Berwaldia schaefernai* isolated from a daphnid. Morphological and molecular data will be presented for this new species and the possibility that microcrustacea may play a role as intermediate hosts in the Hazardia clade of microsporidia from mosquitoes

Contributed paper Monday 17.15 **40**

Diversity of pathogens associated with edible long-horned grasshoppers in East Africa

Alfonce Leonard[1, 2], Fathiya M. Khamis1, Samuel Kyamanywa[2], Sunday Ekesi[1], Komi K. M. Fiaboe[1], Chrysantus Tanga Mbi[1], James P. Egonyu[2], Sevgan Subramanian[1],
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The long-horned grasshopper, *Ruspolia differens* Serville (Orthoptera: Tettigoniidaee) is a delicacy among communities in the Lake Victoria Region of East Africa, especially in Uganda. Their swarming is seasonal, when they impact livelihoods of diverse stakeholders involved in their collection, retailing and wholesale marketing. Recent efforts are focused on their mass rearing to make them available throughout the year. However, streamlining mass-rearing technique and ensuring safety of consumers, requires a detailed understanding of the associated pathogens, which was the focus of this study. Wild-caught populations of *R. differens* were collected from the field in Uganda and a colony initiated at the International Centre of Insect Physiology and Ecology (icipe), Nairobi, Kenya. Samples of dead grasshoppers from the wild and stock colony at icipe were surface sterilized before plating the dorsal and ventral sections of the grasshopper on Potato Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA) to culture associated pathogens. To isolate the bacterial pathogens, grasshopper samples were crushed and plated into nutrient agar and later transferred to lysogeny broth (LB) growth media. DNA extraction was carried out using ISOPLATE Plant and Genomic DNA Kit from BIOLINE for fungi and bacteria, respectively. Polymerase chain reaction (PCR) analysis for fungi and bacteria were conducted using ITS 5 - ITS 4 and 27F-1498R, forward and reverse primers, respectively. Samples were sequenced, edited using BioEdit, and identification done using Basic Local Alignment Search Tool. Major pathogenic fungi identified were *Aspergillus niger, A. flavus, A. tamari, Fusarium equiseti, F. solani, Trichoderma koningii, Purpureocillium lilacinum* and *Alternaria alternata.* Human and veterinary relevant fungi isolated were *Clavispora lusitaniae, Exserohilum mcginnisii, Lichtheimia corymbifera.* Plant pathogens such as *Neopestalotiopsis formicarum, Bipolaris cynodontis, Fusarium solani, Fusarium equiseti, Epicoccum sorghinum* were also isolated. Entomopathogenic bacteria observed included *Serratia marcescens*, while other bacteria of human and veterinary relevance such as *Klebsiella pneumonia*, *Enterobacter cloacae*, *Pantoea* sp., *Proteus vulgaris, P. penneri* and *Providencia* sp. were also identified. This study provides a detailed understanding on the various pathogens associated with long-horned grasshoppers both in the wild and in the laboratory. The implications of this finding on colony sustainability and consumer safety are further discussed.

Tuesday 14 August 2018

Virus Division Symposium

Tuesday 08.00-10.00 Pipeline

Interactions between arboviruses and their vectors

Organisers/Moderators: Karyn Johnson & Rollie Chem

Symposium Tuesday 8.00 **41**

Barriers to arbovirus infection in mosquitoes

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The vast majority of mosquitoes do not transmit arboviruses, and although this may seem surprising at first, it begins to make sense when one examines the complicated process of arbovirus transmission in detail. In order for an arbovirus to be transmitted from one vertebrate host to another, a large number of things have to fall into place, beginning with factors such as mosquito density and feeding behaviour. However, even after being ingested as part of a blood meal, an arbovirus must endure a gauntlet of barriers and immune pathways in the mosquito vector. These include successfully penetrating the mosquito gut, evading immunity, and spreading to the salivary glands, where the arbovirus must replicate and be shed in saliva. In this talk I will focus on two areas of research in the laboratory, the first being the role of apoptosis in controlling arbovirus replication and spread in the mosquito, and the second being the process of midgut escape. We have shown that a version of Sindbis virus expressing a pro-apoptotic gene is strongly selected against in *Aedes aegypti* mosquitoes, indicating that apoptosis is a powerful defense. On the other hand, the process of midgut escape, or how arboviruses exit the mosquito gut, remains a long-standing mystery. The layer of basal lamina surrounding the gut is thought to be impervious to viruses if it is intact. However, in some cases, arboviruses can be detected in the hemolymph within only a few hours of being ingested, suggesting that at least some viruses can leak out of the gut. In contrast, many arboviruses exhibit a midgut escape barrier, where certain virus strains are able to replicate in midgut epithelium, but become trapped and cannot escape. The nature of the midgut escape barrier, and whether it has to do with the level of virus replication in the gut, is the current focus of research in the laboratory. I will discuss some complementary approaches we are using to try to gain a better understanding of whether arbovirus replication in midgut epithelium is actually required for midgut escape.

Symposium Tuesday 8.30 **42**

Mosquito and viral determinants that condition host specificity, tissue tropisms and transmission: spotlight on the flaviviruses

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The flaviviruses (Flaviviridae: Flavivirus) include a number of dual-host species that are transmitted by arthropods and exact devastating global and veterinary health; and the genus includes more recently discovered members that infect only vertebrate, or invertebrate hosts (the insect-specific flaviviruses, or ISFs). The ISFs likely represent the basal state of the genus *Flavivirus*, and the genome of these viruses display some unique features as compared to the dual-host viruses. We reasoned that comparative studies of the insect-specific flaviviruses and dual host flaviviruses could reveal insights into the persistent question of why some flaviviruses infect and cause disease in vertebrate cells, but infect and persist in insect cells without causing overt disease. Further, the ISFs give us a unique model system to study arboviral transovarial transmission (TOT) and filial infection rates (FIR), because these viruses have very high TOT and FIR as compared to the dual-host flaviviruses. We use comparative phylogenetics, infection dynamics and physiology, and chimeric viruses to unravel the underpinnings of the phenotypic outcomes of these infections.

Symposium Tuesday 9.00 **43**

Commensal Viruses of Mosquitoes: Host Restriction, Transmission, and Interaction with Arboviral Pathogens

J**ody Hobson-Peters**[1], Sonja Hall-Mendelin[2], Andrew F van den Hurk[2], Helle Bielefeldt-Ohmann[1], Breeanna J McLean[1], Agathe M G Colmant[1], Cameron E Webb[3], Caitlin A O'Brien[1], Jessica J Harrison[1], David Warrilow[2], Thisun B H Piyasena[1], Alexander A Khromykh[1] and Roy A Hall[1]
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Mosquito-borne viruses are responsible for many important diseases of man and animals. While these viruses usually replicate in both the mosquito vector and a vertebrate host, there has been a recent focus on viral symbionts of mosquitoes that do not infect vertebrates. Interest surrounding these viral symbionts, also referred to as insect-specific viruses (ISVs), stems from observations that they interact

with the mosquito host and interfere with the replication and transmission of viral pathogens.

I will present data on the broad biodiversity of ISVs in Australian mosquitoes, isolates of which represent at least nine viral taxa, including flaviviruses, bunyaviruses, reoviruses, mesoniviruses, negeviruses, iflaviruses, birnaviruses, totiviruses and nodaviruses. Isolation of these viruses has enabled us to investigate ISV transmission, persistence of these viruses in mosquito populations, the mechanisms that restrict their host range to mosquitoes, and their interactions with pathogens transmissible by the same mosquito. I will show data that demonstrate that many ISVs are efficiently transmitted directly from the female mosquito to their progeny via infected eggs, and, moreover, that persistent infection of mosquito cell cultures or whole mosquitoes with ISVs can restrict subsequent infection, replication, and transmission of some mosquito-borne viral pathogens, such as West Nile virus. These data suggest that some ISVs may act as natural regulators of arboviral transmission.

Our more recent investigations have probed the molecular mechanisms of ISV host restriction, through the use of chimeric viruses. These studies provide valuable insights into how ISVs have adapted to an insect-only life cycle and give clues to their evolutionary origins.

Symposium Tuesday 9.30 **44**

Contribution of microRNAs in mosquito-virus interactions

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MicroRNAs (miRNAs) are small (~22 nucleotides) non-coding RNAs that play significant roles in various biological processes, including development, differentiation, apoptosis and immunity. Their function is mainly fine-tuning expression of genes at the post-transcriptional level by interacting with target mRNAs. In recent years, the role of miRNAs in interaction of mosquitoes with microorganisms, in particular viruses, has been demonstrated. In the majority of instances, virus infection leads to changes in the host mosquito miRNA profile. In silico analysis of the target genes has revealed involvement of the target genes in metabolic processes, cellular processes and immunity. The altered host miRNAs might be part of the host anti-viral response to infection, or manipulations by the virus to facilitate its replication, evade host anti-viral responses or optimize its transmission. This presentation will discuss the role of miRNAs in mosquito-virus interactions in the context of host anti-viral responses, evasion of the responses by viruses and facilitation of viral replication.

Contributed papers Maui 1 & 2

Tuesday 8.00-10.00

BACTERIA 1

Moderator**:** Juan Ferre

Contributed paper Tuesday 8.00 45

Discovery of new insecticidal traits from bacteria

Vadim Beilinson and AgBiome team
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Genetically modified crops, particularly corn and soybean, were quickly and widely adopted, and have dominated the U.S. market since late-1990s. There is now a geographically widespread resistance to some of the traits, and resistance is continuing to increase both geographically and in diversity of pests. It is extremely important to discover new traits with new modes of action mechanisms towards agriculturally important pests. AgBiome is addressing this issue by screening of its proprietary bacterial collection of more than 50,000 fully sequences strains to find new potent traits. By employing innovative as well as classic methods, we have built a large and diverse collection of microorganisms and have discovered more than 4,200 putative insecticidal genes that belong to a variety of known and new types of insecticidal gene classes. To date, we have screened a quarter of our discovered genes and found more than 30 proteins showing activity against Lepidopteran, Coleopteran or Hemipteran species. We are currently evaluating these active proteins for efficacy in planta.

Contributed paper Tuesday 8.15 **46**

Do the differences between naturally occurring and GM Cry insecticidal toxins impact to specificity?

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The Cry toxins are a family of crystal-forming protein toxins produced by the bacterium *Bacillus thuringiensis*. Many naturally occurring Cry toxin variants exist and are distinguished on the basis of their protein sequences and toxin specificity. Some Cry toxins, such as Cry1, Cry2, and Cry9 are also used worldwide in GMO crops to protect against insect pests. It is widely assumed that the Cry proteins produced by such crops possess the toxicological spectrum of the natural proteins from which they are derived. Thus Cry toxins naturally active only against coleopterans will remain active only against coleopterans when produced inside a GMO crop. We have catalogued the differences between natural Cry toxins and GM Cry toxins for the most

widely commercialised GMO crops using documents submitted to support GMO approvals, and from patents. Our results show that there is typically an array of chemical and structural differences between natural and GMO Cry toxins. These changes range from loss of crystal structure when produced in plants; deliberate or inadvertent protein truncations; protein additions and mutations; and alterations attributable to protein production or processing in plant tissues. If current mechanistic studies of Cry activation are correct, some of these differences predict a broadened and/or enhanced toxicological activity spectrum for some GMO Cry proteins. In addition, we survey industry tests and other results that appear to show elevated or altered toxicity of GMO Cry toxins. This analysis has potential implications for understanding the effects of GMO insect-resistant crops on nontarget species.

Contributed paper Tuesday 8.30 **47- STU**

Identification of genes involved in the global secretion of entomopathogenic virulence factors in ***Yersinia entomophaga***

Marion Schoof[1], [3], Campbell Sheen[2], Maureen O'Callaghan[3], Travis Glare[1], and Mark Hurst [3]

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The entomopathogen *Yersinia entomophaga*, isolated from a diseased grass grub is a promising candidate for use in biopesticides against pasture pests, such as the New Zealand grass grub Costelytra giveni, the South African black beetle *Heteronychus arator* and other insect species. In liquid culture *Y. entomophaga* releases a wide range of proteins at 25°C into culture including the main virulence factor, the Yen-TC complex. But these proteins are not released at the higher temperature of 37°C. Using random transposon mutagenesis combined with a novel protein screening assay and SDS PAGE, genes involved in protein secretion were identified. Of 4000 screened transposon mutants, 54 mutants showed an altered secretion profile relative to the wildtype bacterium. DNA sequencing of the insertion points identified 54 independent mutants. Several mutants were found to cluster in genes involved in LPS biosynthesis, Rhs4, Quorum sensing and the phoB-like-lysis region. The latter region appears to be essential to the global regulation of secretion in *Y. entomophaga*. The processes controlling secretion of toxin proteins is crucial to the production of a shelf stable economically viable biopesticide.

Contributed paper Tuesday 8.45 **48**

Non-venomous insect sPLA$_2$ and its physiological functions in development and immunity

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Eicosanoids are the oxygenated C20 polyunsaturated fatty acids and mediate various physiological processes in insects. Eicosanoid biosynthesis begins with C20 precursor, arachidonic acid (5,8,11,14-eicosatetraenoic acid: AA). AA is usually released from phospholipids at sn-2 position by a catalytic activity of phospholipase A_2 (PLA_2). Though various PLA_2s classified into 16 gene families (= Groups) are known in biological systems, a few PLA_2s are known in insects. Especially, in lepidopteran insects that are well known in eicosanoid physiology, only two PLA_2s involved in intracellular calcium independent PLA_2 ($iPLA_2$) group are identified. This study reports the first secretory PLA_2 ($sPLA_2$) in the lepidopteran insects. A partial open reading frame (ORF) of PLA_2 was obtained by an interrogation to *Spodoptera exigua* transcriptome. Subsequent 3'-RACE resulted in a full ORF (Se-$sPLA_2$A) encoding 185 amino acid sequence containing signal peptide, calcium-binding domain and catalytic site. A phylogenetic analysis indicates that Se-$sPLA_2$A is clustered with other Group III $sPLA_2$s. Se-$sPLA_2$A was expressed in most larval instars except late last instar. Its expression was inducible to immune challenge and juvenile hormone analog injection. RNA interference of Se-$sPLA_2$A significantly suppressed cellular immunity and impaired larval development. These results suggest that the nonvenomous $sPLA_2$ plays crucial role in immune and developmental

Contributed paper Tuesday 9.00 **49**

Novel mosquitocidal toxins from Clostridia

Estefania Contreras-Navarro, Jianwu Chen and Sarjeet Gill

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New biological insecticides with different mode of actions are needed in order to broaden the range of susceptibility and also to avoid mosquito resistance to presently used biologicals. In our work, we compared the toxicities of two *Clostridium bifermentans* (Cb) mosquitocidal strains and *Bacillus thuringiensis israelensis* against *Aedes aegypti*, *Anopheles gambiae* and *An. stephensi.* The most susceptible mosquito species to Cb *malaysia* (Cbm) was *An. gambiae*. In order to identify the toxic components, we sequenced the genome of the two Cb mosquitocidal strains, a non mosquitocidal Cb wild type strain and a non-toxic Cbm loss of function mutant that we developed using gamma irradiation. Our sequencing data showed that Cb mosquitocidal strains contain a megaplasmid that has two toxin loci. The first one is

composed of Cry16A, Cry17A and two hemolysins. When proteins in this complex were expressed they showed toxicity to *Ae. aegypti*, but not to Anopheles mosquitoes. Interestingly, the second toxin locus shows similarity to other clostridial neurotoxins but with a novel gene organization compared to other similar toxins. We produced different constructs encoding the five protein proteins included in this locus and showed that have toxicity towards *Anopheles* but not towards *Aedes* mosquitoes. Finally we show that the toxin in this complex acts via a novel mechanism of action for mosquitocidal toxins. A discussion of the molecular basis for this toxin's selectivity will be presented.

Contributed paper Tuesday 9.15 **50**

Isolation of cell wall encapsulated or purified Cry5B crystals from asporogenous Bacilli for use as a anthelminthic drug

Ambily Abraham, Deysy Tatiana Pinto Rodriguez, Yan Hu, Hanchen Li, Kelly Flanagan, David Gazzola, Tasia Kellogg, Gary Ostroff and Raffi Aroian
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Soil transmitted helminthiases are some of the most common neglected tropical diseases that affect 1.5 billion people globally, predominantly children and women of child bearing age in poor and developing countries. WHO recommends periodic deworming using small molecule anti-helminthic drugs in endemic areas to counter the health effects of increased worm burdens; abdominal pain, diarrhea, physical and developmental stunting and nutritional deficiency. Such mass administration often leads to the rise of parasite resistance or tolerance and thus a need for a new cure with minimal side effects is imminent. In this context, invertebrate-specific bacterial pore-forming proteins are excellent candidates that have evolved naturally to disrupt host membranes, been widely used as insecticidal pesticides (e.g., Bti, transgenic corn) and also are part of our diet. Crystal protein, Cry5B, from *Bacillus thuringiensis* (Bt) was earlier shown to be efficacious against *Ancylostoma ceylanicum* (hookworm) and *Ascaris suum* (roundworm). However, in these experiments spore-crystal lysates were used to test toxicity to worms. To avoid the dissemination of live spores and the potential enterotoxicity with Bt, we genetically engineered Bt to form a non-sporulating *Bacillus* with Cytosolic Crystal (BaCC). After an inactivation step to kill live vegetative bacteria, the Inactivated BaCC (IBaCC) was tested for bioactivity against Caenorhabditis elegans and hookworms in vitro. Interestingly, the IBaCC bacterial cell ghosts containing Cry5B crystals were highly toxic to the worms, while mutant inactivated *Bacillus* lacking Cry5B had no effect on worms. These results support the continued development of IBaCC as a new cost-effective candidate for veterinary and livestock deworming applications. Formulation development work is ongoing to optimize oral delivery. To isolate Purified Cry5B Crystals (PCC) for pharmaceutical applications, we devised a two-step, simple and scalable process that generated > 90% pure, bioactive Cry5B protein crystals. Taken together, we have developed two forms of Cry5B – encapsulated inactive bacteria (IBaCC) and purified Cry5B crystals with potent anti-helminthic properties. These two new crystal protein production methods can also be extended to other crystal proteins produced in asporogenous hosts.

Contributed paper Tuesday 9.30 **51**

Using an inactivated soil bacterium to kill parasitic nematodes

Yan Hu[1], David Gazzola[1], Hanchen Li[1], Tasia Kellogg[1], Kelly Flanagan[1], Ambily Abraham[1], Martin K. Nielsen[2], Anne Zajac[3], Joseph F. Urban[4], Katherine Petersson[5], Gary Ostroff[1], and Raffi Aroian[1]

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Soil-transmitted helminths (STHs), most notably hookworms, whipworms, and *Ascaris*, are nematodes that infect more than 1.5 billion of the poorest people on Earth and are leading causes of morbidity worldwide. Only one class of de-worming drugs (anthelmintic) is commonly used in mass drug administrations. New anthelmintics are urgently needed to overcome emerging resistance and tolerance, and to produce higher cure rates. Crystal (Cry) proteins, in particular Cry5B made by *Bacillus thuringiensis* (*Bt*) are promising new anthelmintic candidates. Cry5B demonstrates *in vivo* efficacy against multiple GI nematodes in rodents, pigs, dogs and horses. An enormous challenge for STH therapy in the developing world is that treatments must be very cheap (current drugs costs pennies a dose), massively scalable (over 4 billion people are at risk of infection), and have a long shelf life in harsh environment (high humidity, temperature, no cold chain). Here we will update you on *in vivo* efficacy studies against parasites of sheep, dog, horses and rodent models of human disease, as well as our development efforts to produce a deployable version of Cry5B that is cheap, safe, scalable, and stable. These efforts are focused on bacterial engineering, protein expression, fermentation,

downstream processing and formulation. In particular, we will focus on *in vivo* efficacy studies of our new methods to safely delivery Crystal proteins to mammals, namely IBaCC (Inactivated Bacillus with Cytosolic Crystal) and PCC (Purified Cry5B Crystal). We will demonstrate the efficacy of these Crystal protein APIs (active pharmaceutical ingredients) against intestinal nematode parasites of humans and farm animals. In addition, we will discuss our strategies for formulating Crystal proteins for efficacious delivery to the gastro-intestinal tract.

Contributed paper Tuesday 9.45 **52**

Investigation of the role of known insect receptors in mediating the toxicity of nematicidal pore-forming toxins

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Pore-forming toxins (PFTs) are the largest class of bacterial protein toxins and are used for defense against predators such as insects and nematodes. Crucially, secreted toxin monomers must bind to host receptor molecules within the plasma membrane of target cells in order to attain their active oligomeric form and insert into the membrane. Mutant animals that lack these receptors are protected from intoxication, so identifying the receptor(s) for a particular toxin provides key insight into the mechanism of intoxication. Our lab hypothesized that PFTs produced by the bacterium *Bacillus thuringiensis*--might also utilize proteins homologous to known classes of PFT receptors to mediate their toxic effects. We will present the data obtained from testing a panel of *Caenorhabditis elegans* mutants in multiple assays against multiple PFTs. More detailed investigation of a single mutant showed that resistance was specific, as it did not extend to other non-PFT insults. Our results expand our knowledge on the pathology of bacterial pore-forming toxins.

Bacterial Division Symposium
Tuesday 10.30-12.30 Pipeline

Insect resistance mechanisms to Bt

Organisers/Moderators**: David Heckel and Simon Baxter**

Symposium Tuesday 10.30 **53**

Combining deleterious ABC transporter C2 alleles of independent origin causes field resistance to insecticidal Bt toxins

Yang Dong[1], Christopher Ward[1], Zhaojiang Guo[2], Mark Blaxter[3], Neil Crickmore[4], Chris Jiggins[5], Ron Mau[6], Ben Raymond[7], Tony Shelton[8], Robin Shimabuku[6], Youjun Zhang[2], David G. Heckel[9], and **Simon W. Baxter**[1]
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Insecticides help control agricultural pests and reduce the damage they can cause to food crops, yet resistance can cause control failure and crop loss. Resistance to the Bt toxin Cry1Ac was first documented in the Brassica pest *Plutella xylostella*, which carried a small deletion in the insecticide receptor, ABC transporter C2 (ABCC2). Resistance has now been reported in multiple countries, but while experimental crosses between discrete resistant populations demonstrate they share a common genetic mechanism, no direct evidence exists to show that mutations in abcc2 are the likely cause. Here we show that Bt resistance has an ancient origin and arises from different mutations in the abcc2 gene. By sequencing the genomes of seven resistant and three susceptible *Plutella* strains, we find five independent mutations in abcc2 associated with Bt resistance. Strains are typically polymorphic and carry two or more resistance alleles, with isolated populations often sharing identical haplotypes. Molecular dating shows the haplotypes leading to Bt resistance have existed in the standing genetic variation for tens of thousands of years. Using population cage experiments we demonstrate that abcc2 resistance mutations can persist over time, and show Bt toxin has virtually no impact on gene expression profiles of Bt resistant strains. We predict resistance to other insecticides will occur through the fortuitous combination of rare yet ancient alleles.

Symposium Tuesday 10.50 **54**

Bt Resistance in Australian insect pests

Sharon J Downes[1], Tom Walsh[2], Wee Tek Tay[3], Amanda Padovan[2]
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Bt cotton was initially deployed in Australia in the mid-1990s to control the polyphagous pest *Helicoverpa armigera* (Hübner) which was intractably resistant to synthetic chemistries. A conservative strategy was enforced and resistance to first generation single toxin technology was managed. A decade later, shortly after the release

of dual toxin cotton, high baseline frequencies of alleles conferring resistance to one of its components prompted a re-assessment of the thinking behind the potential risks to this technology. Several reviews detail the characteristics of this resistance and the nuances of deploying first and second generation Bt cotton in Australia. Here we explore recent advances and future possibilities to estimate Bt resistance in Australian pest species and define what we see as the critical data for enabling effective pre-emptive strategies. We also foreshadow the deployment of three toxin (Cry1Ac, Cry2Ab, Vip3A) Bollgard 3 cotton, and examine aspects of resistance to its novel component, Vip3A, that we believe may impact on its stewardship.

Symposium Tuesday 11.10 **55**

MAPK signaling pathway trans-regulates differential expression of aminopeptidases N and confers resistance to *Bacillus thuringiensis* Cry1Ac toxin in diamondback mot**h**

Zhaojiang Guo, Shi Kang, Dan Sun, Youjun Zhang
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Rapid evolution of insect resistance seriously threatens the effectiveness of *Bacillus thuringiensis* (Bt) Cry toxins widely adopted in foliage sprays and transgenic crops. Nevertheless, the molecular mechanisms underlying this resistance evolution are still not fully understood, which greatly impedes the sustainability of Bt-based technology. Previously, we found a novel MAPK signaling pathway-triggered resistance mechanism to Bt Cry1Ac toxin in diamondback moth, *Plutella xylostella* (L.). Herein, we further report on Cry1Ac resistance in *P. xylostella* is attributable to differential expression of four midgut aminopeptidase N (APN) genes trans-regulated by the activated MAPK signaling pathway. Genome-wide analysis of M1 aminopeptidase gene family identified 13 classes of PxAPN genes. Expression atlas analyses showed that PxAPN1 and PxAPN3a were dramatically down-regulated in all resistant larvae, while PxAPN5 and PxAPN6 were significantly up-regulated. In vitro heterologous expression, molecular protein docking, in vivo RNAi and CRISPR/Cas9 experiments confirmed that PxAPN1 and PxAPN3a instead of PxAPN5 and PxAPN6 are functional receptors of Cry1Ac toxin. Genetic linkage analysis revealed that Cry1Ac resistance was associated with down-regulation of PxAPN1 and PxAPN3a genes rather than up-regulation of PxAPN5 and PxAPN6 genes. *In vivo* RNAi analysis showed that differential expression of these four PxAPN genes was trans-regulated by the enhanced MAPK cascades thereby conferring Cry1Ac resistance in *P. xylostella*. Collectively, this study further confirms the common role of MAPK signaling pathway for trans-regulating diverse midgut functional genes related to Cry1Ac resistance in *P. xylostella* and greatly advances our understanding of the molecular basis of insect resistance to Bt entomopathogen.

Symposium Tuesday 11.30 **56**

Field-evolved resistance to Bt corn in fall armyworm: mechanism, dispersal and biological implications

Rahul Banerjee[1], Heba Abdelgaffar[1], Omaththage Perera[2], Lucas Hietala[1], Caroline Placidi de Bortoli[1], **Juan Luis Jurat-Fuentes**[1]
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The fall armyworm (*Spodoptera frugiperda*, FAW) is an important polyphagous pest in the American continent and a devastating invasive pest in Africa. Populations of FAW have developed field-evolved resistance to transgenic corn producing the Cry1Fa toxin from *Bacillus thuringiensis* (Bt corn) in multiple locations in North and South America. We previously described an allele linked to resistance to Cry1Fa corn in Puerto Rico (SfABCC2mut) and developed an effective and sensitive genotyping test to detect it in field-collected FAW samples. Since this allele leads to a truncated Cry1Fa-receptor protein, there are important implications related to lack of function of this protein in resistant insects. In this presentation we will share new information on the mechanism of field-evolved resistance to Bt corn in FAW populations from diverse locations, and the dispersal and biological implications of resistance. Information on resistance alleles, their fitness, frequency and dispersal allows for the development of resistance predictive models to improve insect resistance management practices and increase sustainability of Bt crop technology.

Symposium Tuesday 11.50 **57**

Mutations of ABC transporters and Bt resistance in cabbage loopers

Ping Wang[1], Xiaoli Ma[1], Xiaowei Yang[1], Wenbo Chen[2], Wendy Kain[1], Xiaozhao Song[1], Hannah Chu[1] and Zhangjun[2] Fei
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The cabbage looper, *Trichoplusia ni,* has developed resistance to *Bacillus thuringiensis* (Bt) sprays in greenhouse populations. From the Bt resistant populations, *T. ni* strains resistant to Bt toxins Cry1Ac, Cry2Ab and Cry1F were isolated. The resistance traits to the three Cry toxins were all incompletely recessive and the resistant strains did not show significant cross-resistance among the three toxins. With the newly assembled *T. ni* genome, the previously mapped Cry1Ac resistance

locus was localized to a 500 kb region in Chromosome 15. Further fine mapping of the Cry1Ac resistance gene using PCR markers reduced the resistance gene locus to 150 kb containing a cluster of ABC transporter ABCC genes. Expression and mutation of the ABCC2 gene in the Cry1Ac resistant *T. ni* strain were analyzed for the association of the gene with Cry1Ac resistance. The Cry2Ab resistance gene in *T. ni* was localized to Chromosome 17 by whole genome resequencing of female informative backcross families of *T. ni* generated from susceptible and Cry2Ab resistant *T. ni* strains. The Cry2Ab resistance gene in Chromosome 17 was mapped by bulked segregant analyses using both RNA sequencing (RNA-seq) and amplicon sequencing (Amp-seq), and the resistance gene was localized to a region containing two ABC transporter ABCA genes. The ABCA genes were sequenced and their expression was examined to analyze the mutations associated with the resistance.

Symposium Tuesday 12.10 58

Function and role of ATP-binding cassette transporters as a Cry toxins receptor

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Based on analysis of Cry toxin-resistant insect strains, ATP-binding cassette (ABC) transporters have been suggested to be important receptors for Cry toxins. Therefore using the Baculovirus/Sf9 cell expression system, receptor function of *Bombyx mori* ABC family C2 (BmABCC2) was evaluated by cell swelling assays. BmABCC2 expressing cells were susceptible to Cry1Aa, Ab, Ac and Fa toxins, which are active against *B. mori* larvae. In contrast, cells expressing the *B. mori* cadherin-like receptor BtR175 showed lower susceptibility, and cells expressing Aminopeptidase N were not susceptible to these toxins. However, the expression levels of each protein might have not been comparable. Therefore the *Xenopus* oocyte expression system was used, which allows equal amounts of cRNA to be injected. Results with oocytes were qualitatively the same as with Sf9 cells. In both systems, BmABCC2 and BtR175 showed a synergistic effect on pore formation, which so far lacks an explanation. Several molecules assigned to the human ABCC4 clade were introduced into HEK293T cells and the cell-swelling activity of several Cry toxins were observed. ABCC2s and ABCC3s from Lepidoptera, including *Spodoptera exigua* as well as *B. mori*, functioned as receptors for Cry1Aa. In addition, TcABCC4A from *Tribolium castaneum* functioned as a receptor for Cry8Ca. However, Cry1Ca and Da that are active against *B. mori* larvae did not use BmABCC2 and BmABCC3 as receptors. This indicated that ABCCs are widely used by the range of Cry1 to Cry8, but there are Cry1 toxins that cannot use ABCC2 and ABCC3. Affinity of these receptors to the toxins evaluated by surface plasmon resonance (SPR) was correlated to the cell-susceptibility-conferring ability. By the way, BmABCA2 functioned as a receptor for Cry2Ab, suggesting that ABC transporters have a mechanism to function as high potential receptors for Cry toxins. To test whether gate opening of BmABCC2 was required for receptor function, its substrate-excreting activity was abolished. However, BmABCC2 still retained receptor activity. Instead, some extra-cellular loops (ECL) may control binding and susceptibility to Cry1A toxins, as shown by loop replacement experiments.

Contributed papers Maui 1 & 2

Tuesday 10.30-12.30

VIRUS 2

Moderators**:** Martin Erlandson and Monique van Oers

Contributed paper Tuesday 10.30 **59**

Baculoviruses as quasispecies: dynamics and selection *in vivo*.

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The concept of quasispecies has been applied to viruses in which a high mutation rate leads to the generation of multiple strain variants whose diversity is maintained by balancing selection. Quasispecies characteristics are more typically described in RNA viruses, which have a high rate of spontaneous mutation, but the presence of multiple virus variants within single isolates is a characteristic of multiply-occluded baculoviruses, which are double-stranded DNA virus.

In this study, polymorphisms in Baculovirus repeated open reading frame A (BRO-A) region were identified and used to a develop custom 365-base pair 'barcode' sequence within the Helicoverpa armigera Single Nucleopolyhedrovirus (HaSNPV). The diversity of variants within the isolate and their relative abundance were quantified during infection *in vivo* from ultra-deep sequence data in combination with a novel bioinformatics pipeline (MetaGaAP-Py) and more recently-published and widely available software, DADA2.

Change in relative abundance of genotypes was measured during the infection cycle and within occlusion bodies in the initial inoculum and the post-infection cadavers. Evolutionary effects typical of viral quasispecies populations were identified;

weak-negative selection with mutation bias, causing positions within the barcode region to evolve faster than neutral, and a 'drift barrier' that caused the remaining positions to evolve more slowly than neutral, limiting the effects of genetic drift. Baculoviruses, which contain their own DNA polymerase, thus behave as viral 'quasispecies'. The results highlight the potential for rapid selection of strains within isolates during passage and production of baculoviruses biopesticides.

Contributed paper Tuesday 10.45 **60-STU**

Inhibition of adenosine pathway increases host susceptibility to baculovirus infection

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Baculoviruses are circular dsDNA viruses that infect many species of invertebrates, Lepidoptera larva are the most common hosts. AcMNPV, the most commonly studied baculovirus, began to be used for expression of recombinant protein in 1980s. BmNPV are another baculovirus widely produced foreign proteins with BEVS. AcMNPV and BmNPV are highly similar in sequence homology and genome organization, but the host ranges of them are completely independent. BmNPVs replicate and produce viruses in the permissive Bm cell line. By contrast, AcMNPV is weakly expressed in the non-permissive Bm cell. There has been much research on the factors that determine host-specificity between these two baculoviruses. In mammals, for example, cells selective susceptibility, physical barriers, or host defenses that potentially contribute to virus infection of specific tissues have been studied. Therefore, we theorize that insect hosts could possibly influence virus invasion through regulation of physiological pathways. Adenosine, molecules that functions as energy allocation signals, provide energy to induce immune systems when pathogens are infected. We assume that inhibition of adenosine pathways may interrupt energy flows and thus increase host. The manipulation of virus-host specificity can provide a breakthrough for the application of baculovirus in protein expression systems and the development of bio-control agents.

Contributed paper Tuesday 11.00 **61-STU**

Clathrate cage-like apparatus of baculovirus IE2 as a versatile cross-phylum gene transactivation system

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The baculovirus Autographa californica multiple nucleopolyhedrovirus (*Ac*MNPV) is one of the most widely used vectors for protein expression and foreign gene introduction into both insect and mammalian cells. The immediate-early protein IE2 of baculovirus has long been regarded as one of the major transactivators in insect cells upon virus infection. Interestingly, IE2 can also strongly activate several mammalian promoters for gene delivery, but the mechanism underlying this high-level transactivation remains unclear. We found that although IE2 cannot directly bind to DNA, it can still target viral DNA but not host chromosomal DNA through a unique, yet very efficient route. Upon entering into cells, viral DNA is readily captured by the cellular death domain-associated protein (Daxx) and histone H3.3 proteins in the nucleus, both of which are known to be strong suppressors of viral gene expression. Unexpectedly, IE2 interacts with Daxx through IE2 SUMO-interacting motifs, which completely abrogates this strong host cell suppressive mechanism, conversely resulting a novel route to access only its own viral DNA. IE2 then grows into a unique sub-nuclear clathrate cage-like apparatus (CCLA) that functions as a strictly-regulated gene transactivation nanomachinery. CCLAs recruit transactivation-supporting components, such as actin and RNA polymerase II, and physically exclude inhibitory factors such as histone deacetylases (HDACs) to prevent inhibition of gene transcription. After abundant mRNA synthesis, we observed mRNAs together with actin migrating from CCLAs to the cytosol, resulting in high-level protein expression. Our results reveal a novel transactivation mechanism exerted by baculovirus IE2 that functions through a unique nuclear apparatus in insect cells and, interestingly, still functions in distantly related mammalian cells even after 900 million years of divergence from invertebrates.

Contributed paper Tuesday 11.15 **62**

Baculovirus PTP2 functions as a pro-apoptotic protein

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The Spodoptera exigua multiple nucleopoly-hedrovirus (SeMNPV) induces physiological and behavioural changes in its host *Spodoptera exigua*, as well as immunological responses, which may affect virus transmission. Here we show that the SeMNPV-encoded protein tyrosine phosphatase 2 (PTP2) induces apoptosis in *Spodoptera frugiperda* (Sf) 21 cells upon transient expression. Transient expression of a catalytic-site mutant of ptp2 did not lead to apoptosis, indicating that the phosphatase activity of PTP2 is needed to induce apoptosis. We also found that the caspase level (indicator of apoptosis) was higher in cells transfected with the

ptp2 gene than in cells transfected with the catalytic mutant. Adding a caspase inhibitor reduced the level of ptp2-induced apoptosis. Moreover, deletion of the ptp2 gene from the viral genome prevented the induction of apoptosis in *S. exigua* hemocytes. The virus titre and virulence indices (the viral infectivity and the time to death) were not affected by deletion of the ptp2 gene. However, the viral occlusion body yield from *S. exigua* larvae infected with the mutant virus lacking the ptp2 gene was much lower than the yield from larvae infected with the WT wild-type virus. We hypothesize that the observed pro-apoptotic effects of PTP2 are the result of PTP2-mediated immune suppression in larvae, which consequently leads to higher occlusion body yields.

Contributed paper Tuesday 11.30 **63**

The nuclear import mechanism of AcMNPV VP80

Wei Shao[1,2], Lihong He[1,2], Just M. Vlak[3], Fei Deng[1], Hualin Wang[1], Zhihong Hu[1#] and **Manli Wang**[1#]
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The replication and assembly of baculovirus occur within nucleus, however the nuclear import mechanism of baculoviral proteins and host factors involved in this process remains largely known. VP80 is one of viral nucleocapsid proteins that plays an essential role in virus infection. Transient transfection showed that VP80 of Autographa carlifornica multiple nucleopolyhedrovirus (AcMNPV) entered nucleus in the absence of other viral proteins. Two classical nuclear localization signals (NLSs) were predicted in AcVP80, one is monopartite, while the other is bipartite. Mutagenesis analyses showed that both NLSs are of nuclear localization ability and either one is sufficient to mediate the nuclear import of AcVP80. In order to disclose the host NLS receptors that recognize the NLSs of VP80, all the 4 isoforms of Sf9 importin α superfamily (α1, α2, α3 and α7) were cloned. A sensitive method for analysing in vivo protein-protein interactions was used to detect the interactions between VP80 and importin α(s). The results revealed that the full-length VP80 protein could be recognized by α1 and α7. However, α1 only recognizes the monopartite NLS, while α7 specifically recognizes the bipartite NLS, suggesting different cargo recognition specificities of these importin α(s). This study enriched our understanding of nuclear import mechanism of baculovirus in insect cells.

Contributed paper Tuesday 11.45 **64**

Functional analysis of the lysine residues of Autographa californica nucleopolyhedrovirus (AcMNPV) viral ubiquitin

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Many virus-host systems have been shown to utilize the cellular ubiquitination system in multiple aspects of the viral life cycle. Baculoviruses are unique in that they express a gene that encodes a protein that is highly homologous (75% identity) to cellular-ubiquitin (cUbi) and which is called viral-ubiquitin (vUbi). VUbi is highly conserved and homologs have been identified in all Alpha- and Betabaculoviruses, but homologs are not found in Gamma- and Deltabaculoviruses. We recently showed that Autographa californica nucleopolyhedrovirus (AcMNPV) vUbi is required for efficient budded virus (BV) production. In addition, the nucleocapsids of BV, but not occlusion derived virus (ODV) are specifically ubiquitinated by vUbi suggesting it is part of the mechanism that distinguishes nucleocapsid fate. Cubi has been shown to be a signal for multiple cellular functions which include protein degradation, signal transduction, DNA repair and virus budding. Critical to the function of the ubiquitination system is the specific lysine-linkage that is used to form polyubiquitin chains. All seven cUbi lysines are conserved in vUbi. In this study we performed a mutational analysis of all conserved lysines in vUbi to determine which are essential for vUbi function. Using the AcMNPV bacmid system, vUbi was deleted and the virus was repaired with vUbi tagged at the N-terminal with the Myc epitope. In addition, viruses were generated that contained vUbi mutated at each of the conserved lysines and replaced with arginine which cannot participate in the formation of polyubiquitin chains. BV titre for the vUbi deletion virus was reduced by approximately 90%. Surprisingly, no vUbi lysine mutation had a significant impact on BV production. These results suggest that only monoubiquitination of nucleocapsid proteins is required for the production of BV. Total ubiquitination of viral and cellular proteins by vUbi was analysed at 24 and 48 hours post infection. Total viral ubiquitinated proteins showed significant accumulation when K6 and K27 but not K11, K29, K33, K48 and K54 were mutated. K6 and K27 are atypical sites for polyubiquitination and have been shown to be involved in DNA repair mechanisms.

Contributed paper Tuesday 12.00 **65**

Mamestra configurata nucleopolyhedrovirus -B ORF 54, characterization and comparative analysis of homologues in other baculovirus species.

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Two alphabaculovirus species were previously isolated from populations of *Mamestra configurata*, a pest of brassicaceous oilseed crops in western North America. The Mamestra configurata nucleopolyhedrovirus-B (MacoNPV-B) genome was distinguished from MacoNPV-A by the absence of lef-7, the presence of hel-2 and an insertion of homologues of Xestia c-nigrum granulovirus (XecnGV) ORFs 61, 62, 64 and 65. We previously showed that MacoNPV-B ORF 57, a homologue of XecnGV ORF 61, encoded a protein which killed braconid parasitoids associated with noctuid hosts. Another interesting baculovirus gene in this XecnGV cassette is MacoNPV-B ORF 54 (XecnGV ORF 65) which encodes a putative 480 aa protein that has no predicted signal peptide, transmembrane domain or other recognized pfam domains. We have identified homologues of this gene in a number of other MacoNPV-A and –B genomes as well as in an AcMNPV strain isolated from *Trichoplusia ni* larvae. In addition, homologues of this gene are found in a limited number of alpha- and betabaculoviruses from noctuid hosts. Homologues of MacoNPV-B ORF 54 are often found in highly variable regions of alphabaculovirus genomes often adjacent to bro genes, either as a single gene insert or in combinations of the other XecnGV ORFs mentioned above. Although no putative function has been ascribed to this gene, it typically has predicted early or early and late promoter motifs. Here we provide evidence that the MacoNPV-B ORF protein is localized to BV capsid fractions but cannot be detected in ODV using HA-tagged recombinant viruses. Preliminary bioassays with AcMNPV strains with and without MacoNPV-B ORF 54 showed no significant differences in mortality in 1st instar T. ni larvae.

Contributed paper Tuesday12.15 **66**

Identification and characterization of nucleolus localization signal of Autographa californica Multiple Nucleopolyhedrovirus LEF-5 protein

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The baculovirus protein LEF-5 is conserved in all baculovirus. LEF-5 plays a crucial role in directly late gene transcription and virus production. Here, we identified a nucleolus localization signal (Nols) near the carboxy-terminal region of LEF-5 (residues 185 to 204). This region contains three basic-amino-acid-rich clusters, KKKEK (BR1), RKKK (BR2) and KHR (BR3). We constructed multiple mutants by point mutation or HA-LEF5 fusion, respectively. Fluorescent microscopy analysis showed that LEF5-HA and LEF5$^{185\text{-}189}$ could localized to nucleolus, while LEF5$^{197\text{-}200}$ or LEF5$^{202\text{-}204}$ was localized to nucleus of Sf9 cells. LEF5BR2 and LEF5BR3 play the most influential role in Nols localization, and either one of the two LEF5BRs is dispensable if other 2 LEF5BRs are present. LEF5BR mutation not only led to aberrant nucleolus localization but also attenuated protein expression. Collectively, our results revealed the organization of the Nols in LEF-5 and provides insight into the functional role in baculovirus gene expression and virus production.

WEDNESDAY 15 August 2018

Bacterial Division Symposium

Wednesday 08.00-10.00 Pipeline

Insecticidal protein structures

Organisers/Moderators: Mark Hurst and Trevor Jackson

Symposium Wednesday 08.00 **67**

Cryo-EM structure of an insecticidal toxin–ion channel complex reveals the complex molecular basis of allosteric modulation of channel gating

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Spiders are the most successful insect predators on the planet, with >50,000 extant species. Over a period of more than 300 million years they evolved pharmacologically complex venoms that are dominated by disulfide-rich insecticidal toxins. Insect voltage-gated sodium channels are one of the primary targets of these spider toxins. However, in contrast with chemical insecticides such as DDT and pyrethroids, these toxins do not target the pore of the channel but rather allosterically modulate channel gating by interfering with movement of the channel's voltage sensor domains. There has been much speculation, based on indirect experimental evidence, about the molecular mechanism of these gating modifier toxins. We have now solved the first ever structure of a gating modifier toxin complexed with an insect voltage-gated sodium channel. The 2.9 Å resolution cryo-EM structure reveals that the toxin-channel interaction is much more complex than envisaged by any previous model of the interaction, with the toxin making key contacts with both the voltage sensor and pore domains. The structure provides a template for engineering insecticidal sodium channel toxins with enhanced potency and taxonomic selectivity.

Symposium Wednesday 08.20 **68**

Pleurotolysin: a pore forming toxin from the carnivorous oyster mushroom

Stephanie C. Kondos[1], Natasha Lukuyonova[2], Bradley A. Spicer[1], Susan M. Ekkel[1], Helen Saibil[2], **Michelle A. Dunstone**[1]
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Membrane attack complex/perforin-like (MACPF) proteins comprise the largest superfamily of pore forming proteins, playing crucial roles in immunity, venom toxicity, fungal defense and pathogenesis. Moreover the MACPF family share a common evolutionary ancestor with the CDC family of pore forming toxins from Gram positive bacteria. The over-arching mechanism of this MACPF/CDC superfamily involves soluble monomeric proteins that recognise and assemble on the target membrane into a large ring-shaped oligomer. The oligomer can then undergo a massive conformational change to become an integral membrane protein. Here I present the 11 Å resolution cryo-Electron Microscopy (cryo-EM) structure of the two-part fungal toxin Pleurotolysin (Ply), together with crystal structures of both components. This toxin is produced by the carnivorous mushroom, *Pleurotus ostreus* (oyster mushroom), and is suspected to contribute to the killing of nematodes for nutrition/defense specifically targeting sphingomyelin rich domains. These structural data revealed a 13-fold pore 80 Å in diameter and 100 Å in height, with each subunit comprised of a PlyB molecule atop a membrane bound homo-dimer of PlyA. The major conformational changes in PlyB include a 70^{o} opening of the bent and distorted central beta-sheet of the MACPF domain, accompanied by extrusion and refolding of two alpha-helical regions (TMH1 and TMH2) into transmembrane beta-hairpins. Moreover, structures of three different disulphide bond-trapped prepore intermediates allow us to propose that the transmembrane regions assemble into beta-hairpins via a top down zippering mechanism. Our most up-to-date data probes how the membrane recognition/binding component (PlyA) binds specifically to sphingomyelin as a V-shaped homodimer. We demonstrate using analytical size exclusion chromatography, multi angle light scattering (MALS) and analytical ultracentrifugation that PlyA exists in an equilibrium between monomer and dimer. We further investigated the putative dimer interface using site-directed mutagenesis. Some mutants caused perturbed haemolytic activity and red blood cell binding phenotypes. One single mutant W6E causes a substantial loss in membrane binding postulated to be due to the introduction of electrostatic repulsion. These results support the model for pleurotolysin and suggest that PlyA dimerisation occurs prior to membrane binding and allows us to modulate the mode of membrane binding and therefore targeting cell surfaces.

Symposium Wednesday 08.40 **69-STU**

Lessons from the vertebrate immune system: how the Membrane Attack Complex shoots an evolutionary moving target

Bradley A. Spicer[1], Ruby HP Law[1], Charles Bayly-Jones[1], Tom T. Caradox-Davies[3], Paul J. Conroy[1], Susan M Ekkel[1], Natalya Dudkina[2], James C. Whisstock[1], Helen Saibil[2], Michelle A. Dunstone[1]
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The MACPF/CDC pore forming protein family spans all kingdoms of life including well-characterised pore forming toxins from pathogenic bacteria (CDCs), carnivorous mushrooms (pleurotolysin) and fish venoms (stonustoxin). As well as toxins, the MACPF/CDC family includes the key immune effectors with pore forming activity; perforin and the Membrane Attack Complex (MAC).Archetypical pore forming protein complexes rely on dedicated membrane binding domains to recognise and bind the target lipid membrane. The MAC is an effector of the complement pathway in the vertebrate immune system that is able to assembly and punch holes on an incredibly wide range of bacterial, protozoa and parasites cell membranes. Notably, the MAC can target nematodes, such as roundworms (*Trichinella spiralis* larvae, *Haemonchus contortus*), flatworms, such as schistosomes (*Schistosoma mansoni*), *Echinococcus granulosus, Taenia taeniaeformis* and protozoans such as malaria. In other words, the MAC has evolved to assemble on any lipid membrane surface which is in direct contrast to all other pore forming protein systems that use dedicated ancillary domains to specifically recognise a target membrane. The MAC membrane-independent assembly system is an evolutionary solution for a slow evolving vertebrates to overcome fast evolving pathogens. This may provide lessons for ways to overcome pest resistance to pore forming toxins. Here we present the structural studies of the complement C9 protein which allows us to understand how the MAC has evolved a membrane-independent assembly pathway. This includes the X-ray crystal structure of the key pore forming component together with atomic resolution SP cryo-EM structure of the pore. This structural model shows how the MAC can assemble without target specificity and could be used to design membrane independent pore forming proteins.

Symposium Wednesday 09.00 **70**

Cryo-EM structures of the pore-forming ABC toxin from *Yersinia entomophaga*

Sarah J Piper,[2] Lou Brillault,[1,2] Rosalba Rothnagel,[2] Tristan I Croll,[3] Joseph K Box,[1] Sebastian Scherer,[4] Kenneth N Goldie,[4] Sandra A Jones,[5] Femke Schepers,[6] Jason N Busby,[7] Julie E Dalziel,[8] J Shaun Lott,[7] Ben Hankamer,[2] Henning Stahlberg,[4] Mark RH Hurst,[5] **Michael J Landsberg**[1,2]
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ABC toxins are tripartite protein toxin complexes secreted by pathogenic bacteria that prototypically contain three distinct proteins subunits encoded by tc genes. The A subunit, usually encoded by a large, tca-like gene, is responsible for membrane binding and confers host specificity. The B and C subunits (encoded by tcb-like and tcc-like genes) together form a cocoon like structure that encapsulates a highly potent cytotoxin that is encoded by the 3' end of the tcc-like gene. The mechanism by which the toxin is delivered to targeted cells has not been conclusively demonstrated, but is thought to share at least some similarity with other toxin-translocating pore-forming proteins such as the B. anthracis toxin, which delivers its cytotoxin via a transmembrane pore following receptor-mediated endocytosis. We have recently determined cryo-EM structures of the ABC toxin from *Yersinia entomophaga* (YenTc) which is unusual in that its A subunit is encoded by four genes, two of which appear to represent a tca-like gene split into two open reading frames, as well as two chitinase-encoding genes that are functional components of YenTc. Our structures reveal that while the overall fold of the YenTc pore is similar to the previously characterised PTC3 toxin - suggestive of a conserved pore-forming apparatus fold across the ABC toxin family - a number of key structural differences are apparent that are likely to play important roles in differential regulation of cellular recognition and membrane pore formation.

Symposium Wednesday 09.20 **71**

Insights into the cellular recognition patterns of YenTc, an insecticidal pore-forming toxin

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Bacterial ABC toxins are virulence factors expressed by a number of insect and mammalian pathogens. They are large (>2 MDa) tripartite protein complexes that "punch" holes in the insect midgut cell membrane and deliver a lethal cytotoxin. Some members of the *Photorhabdus* & *Xenorhabdus* genera are nematode-associated pathogens while species of the *Serratia* & *Yersinia* genera are direct pathogens of insects. We have investigated the structure and target specificity of YenTc, an orally available ABC toxin expressed by *Yersinia entomophaga*, a pathogen of the New Zealand grass grub (Coleoptera Scarabaeidae), found in soil. We determined cryo-EM structures of the pre-pore and pore-forms of YenTc. These structures revealed an overall topology of the A subunit (contacting the insect membrane) similar to that of known ABC toxins. However, at the level of amino acid sequence and structure, YenTc shows striking differences in its receptor binding domains. We identified novel "leg & feet" domains in our structure that are likely to be involved in contacting the membrane and the presence of chitinases enzymes that form part of the cell surface recognition mechanism. Extensive screening of candidate cell surface receptor motifs has been undertaken, and these results provide clues as to potential cellular recognition mechanisms. Understanding the specific target-cell recognition patterns of ABC toxins could aid their engineering into selective insecticidal agents.

Symposium Wednesday 9.40 **72**

Insecticidal ABC toxin complexes encapsulate a variety of toxins

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ABC toxins are large proteinaceous complexes secreted by a variety of soil bacteria against insects and are active when ingested or delivered into the haemocoel by a nematode vector. These complexes typically contain three main components: TcA forms a pentameric assembly responsible for binding to the target cell and injecting the cytotoxin, while TcB and TcC together form a heterodimer that binds to the TcA pentamer. The C-terminal of TcC contains a cytotoxic domain that is highly variable from species to species, and many bacteria contain multiple copies of the TcC gene with different C-terminal domains. We have studied the ABC toxin complex from the New Zealand soil bacterium *Yersinia entomophaga*, which is highly virulent against a variety of coleopteran and lepidopteran species. We determined the structure of the BC sub-complex by X-ray crystallography, revealing an unprecedented hollow shell formed from a spiral of β-sheet that surrounds and encapsulates the cytotoxic C-terminal domain. Here we show that *Y. entomophaga* contains at least three distinct TcC genes, one of which is at a distant genomic location to the ABC toxin locus, and that all three of these are capable of forming a complex with TcB. We describe the structure of a previously unknown C protein from *Y. entomophaga* in complex with the B protein. We show that the "cargo" is an ADP-ribosyltransferase toxin that is encapsulated in an unfolded or disordered state, with limited areas of local order stabilised by the chaperone-like inner surface of the BC shell. Results elude to a single YenTc delivery vehicle that can deliver three different toxin TcC payloads: two cytoskeleton-disrupting toxins (likely a Rho-activating toxin and an actin-modifying toxin), and a predicted nucleic acid deaminase. This remarkable structure demonstrates how such a wide variety of "cargo" proteins can be delivered by a common mechanism, and suggests that these complexes would be amenable to modification by replacing the "cargo" protein with any other protein of similar size.

Contributed papers Maui 1 & 2

Wednesday 8.00-10.00

MICROBIAL CONTROL 1

Moderator**:** Dietrich Stephan

Contributed paper Wednesday 8.00 **73**

A novel biopesticide to control black beetle in pasture

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African black beetle, *Heteronychus arator* (Scarabaeidae), is an exotic pest of pastures in northern New Zealand that has no registered insecticides for its control. Adult beetles are very mobile, which poses a challenge for field plot trials. A novel biopesticide bait is under development with the bacterium, *Yersinia entomophaga*, as the active ingredient. In October 2015, baits were applied at a rate of 70 kg/ha to 40 x 40 m plots on three Waikato dairy farms. Adult beetle activity was monitored using pitfall traps (13 traps per plot, 3 treated and 3

untreated plots per farm). Traps were checked every 2-4 days for about two weeks after treatment and all live beetles were incubated to check for mortality after collection. In January 2016, soil samples were collected to measure the impact of bait treatments on subsequent larval populations. Beetle mortality ranged from 60-80% after bait application. This led to a 30% reduction in larval numbers in the treated plots. This is the first evidence of success using a biopesticide against this challenging pest.

Contributed paper Wednesday 8.15 **74**

Metarhizium anisopliae infection reduces Trypanosoma congolense reproduction in Glossina fuscipes fuscipes and its vector competence

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Trypanosomiasis management strategies continue to rely on control of the vector that include traps, chemical insecticide-impregnated targets, mass trapping, 'pour on' formulation applied to cattle, repellent collars and sterile insect technique. The need for new strategies to control and possibly eradicate trypanosomiasis cannot be over-emphasized. The potential of the entomopathogenic fungus, *Metarhizium anisopliae* ICIPE 30 in suppressing tsetse fly populations was recently demonstrated in the field using autodissemination devices mounted on pyramidal traps. Here, we report on the effects of infection of tsetse fly, *Glossina fuscipes fuscipes*, by M. anisopliae isolate ICIPE 30 wild-type strain (WT 30) and green fluorescent protein-transformed strain (GZP-1) on the ability of the flies to harbor, acquire and transmit the parasite *Trypanosoma congolense* using molecular approaches. Three days following exposure of flies to treatments, parasite concentration in the control was $4.6 \pm 0.1 \times 10^5$ ml^{-1} while it was significantly reduced in GZP-1 ($1.3 \pm 0.7 \times 10^5$ ml^{-1}) and WT 30 ($8.3 \pm 0.6 \times 10^4$ ml^{-1}) treatments. Five days post-fungal infection, the concentration of *T. congolense* parasite was $1.7 \pm 0.8 \times 10^5$ ml^{-1} in the control as compared to $4.2 \pm 0.5 \times 10^4$ ml^{-1} in GZP-1, while no parasite was observed in WT 30-treated flies. As the days progressed, quantitative PCR showed significant drop in *T. congolense* titers in the flies infected with fungi ($F = 24.42$; $P < 0.0001$) as compared to the control. Flies in the control treatment continued to acquire *T. congolense* while no parasite acquisition was observed in both fungus treatments on day 4. Through scanning electronic microscopy, germinated spores could be observed at nearly all parts of the surface of the insect body from day 2 with no preferential sites. Cross sections of 7-day post-fungal exposure samples of flies showed possible point of interaction between the fungus and the parasite as the intricate fungal mycelial network was seen on and within the haemocoel. This study demonstrated that fungal infection by *M. anisopliae* affects the multiplication of *T. congolense* in *G. f. fuscipes* and thereby its competence to acquire and transmit the trypanosome parasite.

Contributed paper Wednesday 8.30 **75**

NoVil: The hunt for weevil control

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The pepper weevil (PPW), *Anthonomus eugenii,* from Mexico origin, is mainly an insect pest of cultivated chili and sweet pepper (*Capsicum* spp.) but can also reproduce on several Solanum spp. Oviposition and feeding punctures on fruits and blooms by adults can reduce fruit quality while high populations can defoliate plants and prevent fruiting. Up to 90% fruit loss has been reported in untreated experimental plots. On the other hand, cranberry weevil (CBW), *A. musculus*, is native to North America and has a wide host range, besides cranberry, as its name naturally suggests. It causes damage by laying eggs in developing flower buds, which are then fed upon by the weevil larvae. Chemical insecticides are commonly used to control both weevil species but are difficult to control. CBW has developed resistance to some of the broad-spectrum insecticides. Research was conducted to identify isolates of entomopathogenic fungi (EPF) that could be developed as mycoinsecticides for the control of PPW and CBW. Fungal isolates including *Metarhizium robertsii*, Isaria fumosoroseus and *Beauveria bassiana* were screened against both PPW and CBW adults. *M. robertsii* isolate CPD6 (NoVil) caused over 90% mortalities by mycosis in PPW and hibernating-population of CBW within 4 days and had the lower lethal concentration values. However, summer population of CBW was less susceptible to fungal infection, with NoVil achieving mortality of 97% after 9 days. The efficacy of NoVil was subsequently tested in hoop house against PPW. Application of emulsifiable formulation on pepper plants artificially-infected with PPW resulted in 75% reduction of weevils over the control, which was comparable to chemical insecticide Flagship. There was also a significant reduction in the number of oviposition punctures ($P=0.04$) and adult emergence ($P=0.001$) in NoVil-treated plants as compared to the control. NoVil persisted for up to 6 days in hoop house

experiment, causing mortality of 100% of insects exposed to leaf samples collected at 0, 3 and 6 days after treatment. NoVil was able to endophytically colonized root (28%) and stem (2%) of pepper plants following seed inoculation. NoVil has therefore the potential to control PPW and CBW and is being considered for registration.

Contributed paper Wednesday 8.45 **76-STU**

Cancelled

Contributed paper Wednesday 9.00 **77**

The 'MycoHarvester': a device for optimising mycopesticide storage and formulation

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A technique for separating powdery conidia of beneficial fungi from substrates such as rice grains was first developed more than 20 years ago. The cyclonic system underwent various modifications before standardising on the geometry used in the present 'MycoHarvester': which is made in autoclavable, food-grade stainless steel (www.mycoharvester.info). The particle size spectra of separated products have now been characterised for quite a wide range of fungi including: *Beauveria*, *Isaria* (*Paecillomyces*), *Purpureocillium* and *Metarhizium* spp., with other beneficial fungi including a number of relatively newly-described plant disease antagonist species in the *Trichoderma harzianum* group. The initial requirement (by the International LUBILOSA Programme) was for a very high particle size specification in order to prepare formulations of *Metarhizium acridum* for ultra-low volume (ULV: typically <1 litre/ha) applications against locusts and grasshoppers. This included elimination of large (>100 µm) particles, which can block spray nozzles, and maximising (preferably to >99%) the volume in the 1-60 µm size range; for optimising suspensibility, obtaining >80% yield as particles up to say 15 µm (depending on the isolate) is highly desirable. Parallel research on *Beauveria* and *Metarhizium* isolates established that survival of conidia is greatly increased by drying before storage; thus concentrating spores for efficient, subsequent desiccation (and elimination of potentially hygroscopic substrate particles) is also essential. Other important features include fast, cost effective processing of substrates and operator safety: any escaping spore dust is sucked into the inlet with this machine. From 2017 a new MycoHarvester version 6 (MH6) has been fitted with an upgraded fan unit powered by a small industrial-type 3-phase motor: using an inverter to connect to single-phase supplies as required. It replaces previous models, designed primarily for research, and is now more compatible with small-medium scale operational production – for which there is increased interest. Optimising mycopesticide product storage appears to be at least as important a factor to clients as formulation compatibility with standard application techniques. I will discuss the roles and standards required for small-medium scale production in contemporary, especially tropical, pest management.

Contributed paper Wednesday 9.15 **78-STU**

Yeast-baculovirus synergism: Investigating mixed infections for improved management of the false codling moth, *Thaumatotibia leucotreta*

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Thaumatotibia leucotreta (false codling moth) is an indigenous pest of the citrus industry in southern Africa. The pest is highly significant as it impacts negatively on the export of fresh citrus from South Africa to international markets. To control *T. leucotreta* in South Africa, an IPM programme has been implemented. One component of this programme is the baculovirus, Cryptophlebia leucotreta granulovirus (CrleGV-SA). It has previously been reported that there is a mutualistic association between *Cydia pomonella* also known as codling moth, and epiphytic yeasts. Laboratory assays and field trials show that combining yeast with Cydia pomonella granulovirus significantly increased larval mortality. Consequently, we are determining which species of yeast occur naturally in *T. leucotreta* larvae and examining whether any of these yeasts, when combined with CrleGV-SA, increase larval mortality. Navel oranges infested with *T. leucotreta* larvae were collected from orchards in South Africa and analysed for the presence of yeast. Four yeasts were isolated from *T. leucotreta* larvae and identified down to species level via PCR amplification and sequencing of ITS region and D1/D2 domain of the LSU. A yeast preference assay was conducted on female *T. leucotreta* moths to examine whether any of the isolated yeast species affected their oviposition preference. Significantly more eggs were deposited on Navel oranges inoculated with one of the yeast species, compared to the other treatments, indicating that it was a female attractant or oviposition stimulant. A detached fruit bioassay was then performed to evaluate the efficacy of mixing this yeast with CrleGV-SA. Although an increase in larval mortality was observed between CrleGV-SA being applied alone and the yeast/virus mixture, this result was determined not to be statistically significant. Currently we are expanding the bioprospecting process to other geographically

distinct citrus producing regions such as the Western Cape and Limpopo provinces. This may lead to the identification of unique yeast isolates that elicit stronger responses in *T. leucotreta* than those isolated thus far. Additionally, detached fruit bioassays are being conducted with varying yeast concentrations and the addition of molasses. The inclusion of molasses and lowering the yeast concentration may result in better mortality rates.

Contributed paper Wednesday 9.30 **ADDED**

Where do *Isaria fumosorosea* and *Burkholderia rinojensis* fit in with chemical and biological pesticides for zucchini pest management?

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Zucchini in California has multiple arthropod pests including aphids, thrips, whiteflies, and mites. A field study was conducted to evaluate the efficacy of the chemical pesticides flupyradifurone (Sivanto) and sulfoxaflor (Sequoia), the entomopathogenic fungus *Isaria fumosorosea* (PFT-97), the bacterium *Burkholderia rinojensis* (heat-killed) (Venerate XC), the spider venom peptide GS-omega/kappa-Hxtx-Hv1a (Spear C), and an experimental botanical extract at different concentrations. The efficacy of these materials varied against different pests following two weekly applications. Chemical pesticides provided the best control followed by the spider venom peptide, the botanical extract, the bacterial compound, and the entomopathogenic fungus

79-STU Cancelled

80-STU Cancelled

Contributed papers Maui 3

Wednesday 8.00-10.00

VIRUS 3

Moderators: Vera Ros and Robert Possee

Contributed paper Wednesday 8.00 **81**

Use of whole genome sequences for baculovirus species demarcation and taxonomy

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Advances in next generation sequencing (NGS) techniques and easy access to these technologies let to a dramatic increase of entirely sequenced, annotated and published genomes within the virus family Baculoviridae. Up to spring 2018, a total of 172 fully sequenced genomes of different lepidopteran, hymenopteran and dipteran baculovirus isolates have been deposited at GenBank; many of these isolates are without clear taxonomic status. Previously, Kimura 2-parameter nucleotide distances of only three baculovirus genes, namely the polyhedrin/granulin (polh/gran), late expression factor 8 (lef-8) and late expression factor 9 (lef-9) gene, have been used as a tool for species demarcation of lepidopteran-specific baculoviruses. Same species are identified by pairwise distances below a threshold of 0.015 and are considered as possibly belonging to the same species with a distance between thresholds of 0.015 and 0.05. In the present study, this species demarcation criterion was newly defined under by considering all known baculovirus core genes, which represent the established standard information for baculovirus phylogeny. In total, a bioinformatic analysis of 38 core genes from 172 baculovirus genomes resulted in 5405 comparisons of genome distances. By this comprehensive comparison covering all available baculovirus genomes we demonstrate the robustness of the present classification and improve the thresholds for baculovirus species demarcation by calculating genetic distances that are based on the highly conserved baculovirus core gene set.

Contributed paper Wednesday 8.15 **82**

Baculovirus invasion of the lepidopteran central nervous system

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Baculoviruses are known to induce behavioural changes in their caterpillar host. Infected caterpillars show enhanced mobility (hyperactivity) and climb to the top of plants prior to death (tree-top disease). These changes are thought to spread the virus over a larger area, enhancing virus transmission. While few viral genes have been studied for their role in inducing these behavioural changes, host pathways and processes involved have hardly been explored. Since behaviour is primarily regulated via the host central nervous system (CNS), viruses possibly affect host behaviour through affecting or invading the host CNS.

However, little information is available on the morphology and function of the CNS of lepidopteran larvae. Here, we present a 3D model of the brain of third instar *Spodoptera exigua* larvae, obtained by immunolabelling of serotonergic cells. Additionally, we explored the distribution of serotonergic neuron cells in the larval brain. As a next step, we investigated whether baculoviruses enter the larval brain, exploring virus localization and temporal invasion patterns. We show that the baculovirus Autographa californica multiple nucleopolyhedrovirus (AcMNPV) enters the brain of third instar *S. exigua* larvae at two days post infection at the lateral sides, and further spreads to specific cells at three days post infection. Electron microscopy results confirmed that AcMNPV replicates in the brain. Our results contribute to an increased understanding of viral invasion of insect brains and provide useful information for future studies on the mechanism of viral manipulation of host behaviour.

Contributed paper Wednesday 8.30 **83-STU**

Whole genome analysis of a baculovirus isolated from a persistently infected insect cell line

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Autographa californica nucleopolyhedrovirus (AcMNPV) normally causes lethal infection in insects. Nevertheless, many insects harbour persistent, non-lethal virus infections without obvious harm. Studying this state in vivo is difficult. A partial solution is provided by a clonal cell line (C20) derived from *Trichoplusia ni* (Hi5) cells that is persistently infected with an AcMNPV p10 deletion mutant. This cell line has been in culture continuously for over seven years (>150 passages), produces low levels of budded virus (BV; $<10^5$ pfu/ml), can survive for up to one month before requiring sub culture and is largely resistant to virus superinfection. Whole virus genome sequencing suggested significant deletions in p95 (orf83; capsid-associated protein), p74 and egt/DA26 (orf16; putative capsid-associated protein) that will be analysed further. The possible relation between these mutations and the C20 virus phenotype will be investigated. However, when amplified in *Spodoptera frugiperda* cells near normal infectivity titres are obtained. Intriguingly, although budded virus productivity by C20 cells is low, the virus has a defective fp25. These initial results provide us with an insight into how a normally lethal baculovirus infection may adapt to survive in a non-lethal state and pave the way for future study of persistent virus infections in vivo.

Contributed paper Wednesday 8.45 **84-STU**

Transcriptome analysis of an insect cell line harbouring a persistent baculovirus infection

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The C20 cell line is a clonal derivation of *Trichoplusia ni* Hi5 that supports continuous replication of a low-level infection of AcMNPV. It provides a unique opportunity to study the mechanisms of baculovirus persistence *in vitro*. It exhibits unusually prolonged viability and continuous production of low levels of infectious budded virus. Moreover, the persistent virus genome has only undergone minor gene deletions. These results indicate a fine balance between the host cell and the replicating virus, which is very different from an overt virus infection. To gain a detailed picture of viral and host gene expression in C20 cells, strand-specific RNA-sequencing was performed on C20 cells and C20 cells challenged with AcMNPV. Gene expression was compared to AcMNPV-infected Hi5 cells. Analysis revealed that all AcMNPV genes were expressed in C20 cells at low levels but without distinct temporal phases or shut down of host gene expression. We also examined the effects on a variety of specific cellular pathways, including stress responses. Because persistent viral infections of insect cells are common in nature and important in biotechnology, this model system will be valuable for discovering and understanding the specific features of the virus-host interaction that permit and maintain persistent infection.

Contributed paper Wednesday 9.00 **85-STU**

Integration of transcriptomics and metabolomics reveals a role of cellular methylation process during *Bombyx mori* nucleopolyhedrovirus infection

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Baculovirus infection causes a global shutoff of host protein synthesis and gene expression in host insect cells. This shutoff is considered to maximize the cellular resources available for the virus and to

ensure efficient virus propagation. In contrast, basic functions including protein synthesis and cellular metabolism are essential for virus propagation so that a portion of the host genes should escape from the shutoff. Previous studies identified several escaping genes, such as a chaperone gene, heat shock cognate 70 (hsc-70), from various baculovirus-host combinations, but a common list of the escaping genes has not been made. In this study, we obtained transcriptome and metabolome data from *Bombyx mori* BmN-4 cells infected with *Bombyx mori* nucleopolyhedrovirus (BmNPV). Transcriptome analysis revealed a global downregulation of host genes with a small cluster of the escaping genes. This cluster included hsc-70, supporting the validity of our selection criteria. The majority of these escaping genes encode proteins related to protein synthesis, mainly ribosomal proteins, which coincide with the hypothesis that the escaping genes are required for maintaining the basic cellular functions. We found that an S-adenosyl methionine (SAM) synthase gene escaped from the shutoff. SAM is a cellular methyl donner and converted into S-adenosyl homocysteine (SAHC) by transferring the methyl group to its substrates. Metabolome analysis revealed that both SAM and SAHC were highly accumulated in BmNPV-infected cells compared to those in uninfected cells. BmNPV encodes a homologue of SAM-dependent methyltransferase, Bm57 (Ac67), whose function during BmNPV infection is unknown. Bm57-deletion BmNPV mutant produced fewer occlusion bodies and exhibited a slow-killing phenotype in *B. mori* larvae. On the other hand, host SAM-dependent methyltransferase genes were downregulated during BmNPV infection. These results suggest that the cellular methylation process is selectively maintained by cooperating with host and viral factors in BmNPV-infected cells, facilitating BmNPV propagation. This is the first report showing the importance of cellular methylation process in baculovirus infection.

Contributed paper Wednesday 9.15 **86**

Structural Proteins of the Aedes sollicitans Nucleopolyhedrovirus (AesoNPV)

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Complete genome of the nucleopolyhedrovirus of *Aedes solicitans* (AesoNPV) was sequenced and assembled using Illumina short reads and the accurate assembly was verified by sequencing nine overlapping PCR amplicons ranging from 9.4 to 10.8 Kbp with MinION long reads. The 85,135 bp genome contained 110 open reading frames (ORF) coding for 60 or more amino acids of which 45 were similar to polypeptides produced by *Culex nigripalpis* NPV (CuniNPV). Proteins of the occlusion derived virions (ODVs) and whole occlusion body (OB) preparations of the AesoNPV were separated on SDS-PAGE and analyzed using liquid chromatography-mass spectrometry of gel separated protein fragments (GeLC-MS/MS). Oligopeptide sequences generated by GeLC-MS/MS analysis were searched against a database of 206 polypeptides derived from non-nested open reading frames greater than 150 bp derived from the complete AesoNPV genome. A total of 24 and 58 proteins, including the major occlusion body protein, were identified in the ODV and OB protein preparations, respectively. Of the 24 polypeptides identified in the ODV analysis, five were unique to AesoNPV and no counterparts were found in public databases. The remaining 19 polypeptides had similarities to CuniNPV. Of the 58 polypeptides identified in the OB analysis, 26 were unique to AesoNPV. Total OB protein analysis also identified five additional polypeptides that are known to be structural proteins of ODVs of CuniNPV.

Contributed paper Wednesday 9.30 **87**

The Se301 cell Aggregation induced by Spodoptera exigua multiple Nucleopolyhedrovirus

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Spodoptera exigua multiple nucleopolyhedrovirus (SeMNPV) and Se301 cells were used to investigate the signal transduction pathway and the biological significance of the baculovirus-induced cell aggregation. By using the aggregation index analysis, the SeMNPV-induced Se301 cell aggregation was found to begin before 24 h post-infection (p.i.) and the aggregation extent increased significantly at 48 h p.i. Infection of Autographa californica multiple nucleopolyhedrovirus (AcMNPV) did not cause Se301 cells to aggregate, indicating that the SeMNPV-induced Se301 cell aggregation may be species-specific. In an RNA interference assay, the treatment of dsRNA for rho1 suppressed the SeMNPV-induced Se301 aggregation at 48 h p.i. The Rho-associated protein kinase (Rok) is an effector of Rho1 and the Rok inhibitor, Y-27632, decreased the extent of the SeMNPV-induced Se301 cell aggregation in a concentration-dependent manner. These results indicated that the Rho1/Rok pathway participates in the SeMNPV-induced Se301 cell aggregation. The progeny virus production upon Y-27632 treatment was also detected. At a high multiplicity of infection (MOI), 5 $TCID_{50}$/cell, virus production was not affected by the treatment of Y-27632. In contrast, at a low MOI, 0.5 $TCID_{50}$/cell, the virus production

from the Y-27632-treated cells significantly decreased at 24 and 48 h p.i. This result indicated that the Rho1/Rok pathway and/or cell aggregation may participate in the spread of SeMNPV among cells.

Contributed paper Wednesday 9.45 **88**

Uncleaved GP64 signal peptide of Bombyx mori nucleopolyhedrovirus is required for cell membrane localization and its special dependence to cholesterol recognition amino acid consensus

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GP64 belongs to class III membrane fusion protein, which mediate Budded Virus(BV) fusion with host cells or nonhost cells. Although Bombyx mori nucleopolyhedrovirus (BmNPV) genome is over 90% identical to that of Autographa californica multiple nucleopolyhedrovirus (AcMNPV), the host ranges of these two viruses have essentially no overlapping, however, substitution of BmNPV GP64 with AcMNPV GP64 led BmNPV entry into non-permissive cell nucleus. Signal peptide (SP) is cleaved by signal peptidase after cotranslational translocation for membrane protein except few special proteins. BmNPV GP64 sequence contains a longer SP than AcMNPV GP64. We found that Myc-tagged signal peptide in GP64 was detected in mature BVs, it is unclear if this result from the Myc-tag insertion breaks the signal peptide characteristic. Here, we verified that further SP of BmNPV was not cleaved with truncated SP of GP64 constructs and consequential infectivity of recombinant viruses with different SP. The bioinformatics prediction indicated that the full-length SP of GP64 includes n-region (SPI) and h-region and c-region (SPII), and transient expression of GP64 localized to cell membrane, and neither SPIGP64 nor SPIIGP64 localization of cell membrane were indicated by immunofluorescence assay, recombinant virus with SPIGP64 abolished viral infectivity, however, SPIIGP64 showed similar infectivity with wild type GP64. MS analysis and N-terminus sequencing of GP64 indicated that SP was not cleaved in mature GP64. Consequentially, this uncleaved SP in GP64 determined the special characteristic for BmNPV GP64 structure. Based on AcMNPV GP64 post fusion structure, key amino acids mutants on two fusion loops showed no effect on BmNPV infectivity, which indicated that BmNPV may adapt other different fusion mechanism with that of AcMNPV. BmNPV entry was dependent on host cell membrane cholesterol level; depletion of cell cholesterol blocked virus infection completely. Here, we showed that BmNPV infection depended on *cholesterol* recognition amino acid consensus (CRAC); key amino acid mutant $CRAC_1$ and $CRAC_2$ of GP64 resulted in abortive infection, and $CRAC_3$ mutant virus postponed the survival time of infected larvae. Taken together, our results indicated BmNPV GP64 took a different entry mechanism to mediate by the uncleaved SP, which paved the road for new start of BmNPV entry mechanism

Beneficial Insects Division Symposium

Wednesday 10.30-12.30 Pipeline

Health issues of bee and non-bee pollinators

Organisers/Moderators: Helen Hesketh & Elke Genersch

Symposium Wednesday 10.30 **89**

Viral landscape of *Varroa*-free Australian honey bees

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Honeybee (*Apis mellifera*) health is threatened globally by the complex interaction of multiple stressors, including the parasitic mite *Varroa destructor* and a number of pathogenic viruses. Australia provides a unique opportunity to study this pathogenic viral landscape in the absence of *V. destructor*. We analysed 1,240 *A. mellifera* colonies across Australia by reverse transcription-polymerase chain reaction (RT-PCR) and high-throughput sequencing (HTS). Five viruses were prevalent: black queen cell virus (BQCV), sacbrood virus (SBV), Israeli acute paralysis virus (IAPV) and the Lake Sinai viruses (LSV1 and LSV2), but several viruses were absent in our sampling, including deformed wing virus (DWV) and slow bee paralysis virus (SBPV). HTS also detected multiple known and putatively novel insect virus genomes. Our findings highlight that viruses can be highly prevalent in *A. mellifera* populations independently of *V. destructor* and we propose that a diverse population of these viruses may be representative of a *Varroa*-free landscape. Placing these results in an international context, our results support the hypothesis that the co-pathogenic interaction of *V. destructor* and DWV is a key driver of increased colony losses, but additional stressors such as pesticides, poor nutrition, etc. may enable more severe and frequent colony losses to occur.

Symposium Wednesday 11.00 **90**

The role of Deformed wing virus (DWV) in *Varroa* tolerant honey bee populations and its spread beyond bees into the wider insect community

Laura E. Brettell[1,2], Jessika Santamaria[3], Ethel Villalobos[3], Gideon Mordecai[4,5]; Declan Schroeder[4,6]; Stephen J Martin[2]

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Deformed wing virus (DWV) in association with its vector the parasitic *Varroa* mite has been responsible for large scale honey bee colony losses throughout the northern hemisphere and continues to be a major problem for beekeepers, although isolated tolerant populations of European honey bees do exist. Furthermore, there is now concern not only for honey bees but also for the ecosystem more generally, as it has become apparent that DWV is a multi-host virus capable of infecting a broad range of taxa and in some cases may be pathogenic. The DWV species group is made up of three master variants (types A, B and C) which frequently coinfect individual hosts and readily recombine. However, despite significant research efforts over the past few decades there is still much that is unclear about the role of these different DWV variants and recombinants in disease, transmission and interspecies dynamics. We investigated the role of DWV in two *Varroa* tolerant populations, as well as its spread beyond honey bees into the wider insect community which is likely to occur via the predation of honey bees and consumption of hive materials, but also, perhaps more worryingly, through the sharing of floral resources.

Symposium Wednesday 11.35 **91**

A natural product inhibited the replication and expression of Israeli acute paralysis virus

Yang Sa [1,2], Xu Xiang[1,2], Zhao Hongxia [1,2,3], Deng Shuai [1,2], Chu Yanna [1,2], Yang Dahe [1,2,4], Wang Xinling [1,2], Zhao Di [1,2], Diao Qingyun [1,2], **Hou Chunsheng** [1,2]
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Honeybee is fundamental to supply the pollination service for increasing the agricultural production and biodiversity. Recently in America and European countries, however, honeybee colony went through a large number of losses that has been linked with a RNA virus, Israeli acute paralysis virus (IAPV). Current knowledge about honey bee virus is limited, especially on virulence and pathogenicity of IAPV due to the lack of honey bee virus cell. Thus, it is crucial to construct a reverse genetic system to understand clearly the infection and develop effective drug to control IAPV. For this purpose, we constructed an infectious cDNA of full-length genomic clone of IAPV and rescued it from infected honey bee, and displayed identical phenotypes with wild virus. To further study the effect of natural product (named Q here) on inhibition of IAPV replication, we injected the healthy adult bees with constructed infectious IAPV and investigated the effect of Q application on their survival. Our results indicated that we not only constructed infectious clone of IAPV with virulence but also found one agent based on natural product to control the IAPV infection. Thus, we provided a power tool to study the molecular mechanisms involved in viral genome replication and virus pathogenesis, and found a potent antiviral agent that can be used widely in field. These results pave the way for further study the infection mechanism of honey bee virus as well as for antiviral treatment of bee viruses infected hives in practice. To our knowledge, our study provides the first infectious clone and antiviral agent based on the natural product and established a general model platform for studying the genetic characterization and gene functions of honey bee viruses.

Symposium Wednesday 11.50 **92**

Enhancement of chronic bee paralysis virus levels in honeybees acute exposed to imidacloprid: a chinese case study

Diao Qingyun [1,2#], Li Beibei [1,2#], Zhao Hongxia [3], Wu Yanyan [1,2], Guo Rui [4], Dai Pingli [1,2], Chen Dafu [4], Wang Qiang [1,2,] Hou Chunsheng [1,2*]
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Though honeybee populations have not yet been reported to be largely lost in China, many stressors that affect the health of honeybees have been confirmed. Honeybees inevitably come into contact with environmental stressors that are not intended to target honeybees, such as pesticides. Although large-scale losses of honeybee colonies are thought to be associated with viruses, these viruses usually lead to covert infections and to not cause acute damage if the bees do not encounter outside stressors. To reveal the potential relationship between acute pesticides and viruses, we applied different doses of imidacloprid to adult bees that were primarily infected with low levels (4.3×10^5

genome copies) of chronic bee paralysis virus (CBPV) to observe whether the acute oral toxicity of imidacloprid was able to elevate the level of CBPV. Here, we found that the titer of CBPV was significantly elevated in adult bees after 96 h of acute treatment with imidacloprid at the highest dose 66.9 ng/bee compared with other treatments and controls. Our study provides clear evidence that exposure to acute high doses of imidacloprid in honeybees persistently infected by CBPV can exert a remarkably negative effect on honeybee survival. These results imply that acute environmental stressors might be one of the major accelerators causing rapid viral replication, which may progress to cause mass proliferation and dissemination and lead to colony decline. The present study will be useful for better understanding the harm caused by this pesticide, especially regarding how honeybee tolerance to the viral infection might be altered by acute pesticide exposure.

Symposium Wednesday 12.05 **93**

Evidence of deformed wing virus (DWV) – free honey bee populations in the Pacific region

John M. K. Roberts

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Honey bees are not native to the Pacific region. The European honey bee (*Apis mellifera*) has been introduced for honey production, while the Asian honey bee (*A. cerana*) has spread invasively from Indonesia into Papua New Guinea, Solomon Islands and Northern Australia. In recent investigations of the pathogen status of these bee populations and their parasitic mites we did not detect several important bee pathogens, including the widespread deformed wing virus (DWV). This virus is a major pathogen of *A. mellifera* around the world in association with the parasitic mite *Varroa destructor*. These findings have important biosecurity implications for the region and provide further evidence that DWV is not established in all bee populations.

Contributed papers Maui 1 & 2

Wednesday 10.30-12.30

MICROBIAL CONTROL 2

Moderator: Dietrich Stephan

Contributed paper Wednesday 10.30 **94**

BASF's bio-insecticide portfolio – Nemasys and Velifer – new products for the Australian market

Sarah Anderson[1], Diana K Londono[2], Shaun D Berry[2]

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BASF is working in the development of biologicals-based solutions (nematodes, bacteria, fungi) for seed, foliar and soil applications to expand the current portfolio. The mindset is to extend the window of protection, offer integrated pest management solutions and give other options for resistance management programs. BASF is the world's largest manufacturer of entomopathogenic nematodes (EPNs) and sells six key different nematodes that suppress and/or control important economic pests such as citrus weevil (controlled by the only *Steinernema riobrave* product in the market) and slugs (controlled by the only *Phasmarhabditis hermaphrodita* product in the market). EPNs allow zero re-entry interval/harvest interval with excellent compatibility and performance with chemistry and other biologicals. Another new product Velifer (containing *Beauveria bassiana* PPRI5339), is registered in Canada for greenhouse, vegetables and ornamentals use and will be introduced into the Australia market in early 2019 for use on aphids, mites, thrips, whiteflies.

Contributing paper Wednesday 10.45 **95 STU**

Influence of host nutrition on mixed pathogen interactions: disease outcome and pathogen replication

Pauline Deschodt, Olivia Walker, Alana Breitkreutz, Jessi Ly and Jenny Cory

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Hosts are commonly challenged by multiple pathogen species. Yet, studies on insect-pathogen interactions are mainly considering direct interactions between a single host and a single pathogen. Pathogen interactions within-host is complex. Two pathogens co-infecting a host may compete directly (interference) or indirectly, for resources or via the host immune system. In any case, the host-pathogen dynamic can be altered by the host nutritional intakes. In insects, increased dietary protein can increase survival, even post-infection. However, the role of nutrition in mixed pathogen infections is not known, but is likely to relate to the relative cost of resistance to pathogen groups and trade-offs between immunity measures. Using the cabbage looper, *Trichoplusia ni*, its nucleopolyhedrovirus (TnSNPV) and the entomopathogenic fungus, *Beauveria bassiana*, we asked whether host nutritional intake could alter the outcome of a mixed infection. Firstly, we challenged *T.ni* larvae with either a single pathogen species or two simultaneously, then we reared them on an artificial diet differing in levels of two major macronutrients, protein and digestible carbohydrates (quality) or the total amount of these two macronutrients (quantity). Secondly, we investigated the effect of host plant on mixed pathogen infection, using two *T.ni* host plants,

broccoli and cucumber. Post infection, freshly cut leaves were provided to larvae daily until death or pupation. Broccoli represented a good quality plant compared to cucumber. The results suggest that pathogens respond differently to host nutritional intake. Poor quality diet tends to exacerbate the negative effect of pathogen on host survival. Moreover, in co-infection, the effect of diet composition on host mortality seems to be greater at lower doses. These results are important in our understanding of how pathogen interactions can affect competing species within the host.

Contributed paper Wednesday 11.00 **96**

CRISPR/Cas-mediated gene editing of ATP Binding Cassette Transporter type-A3 in *Spodoptera frugiperda* results in high resistance to Cry2Ab-like protein

John Mathis, Deirdre Kapka-Kitzman, Cathi Clark, Jean Dyer, Joe Zhao, Amit Sethi and Mark Nelson
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Insect protected transgenic maize has been developed based on the insecticidal proteins from *Bacillus thuringiensis* (Bt). However, the Bt proteins in use for lepidopteran control represent a limited number of modes of action (MoAs). Pyramids of Bt proteins have been developed as a way of promoting trait durability to prolong the usefulness of these MoAs, but to be effective each protein in the pyramid must achieve activity by binding to different receptors (target sites) in the insect gut. Identification of Bt receptors helps in differentiating their MoAs, in understanding how resistance might occur, and can lead to potential resistance monitoring tools. We have demonstrated direct interaction of a Cry2Ab-like protein with ATP-binding cassette transporter subtype A (ABC-A) by pulldown assays using midgut tissue from several Lepidoptera. Furthermore, mutations in ABC-A proteins have been linked to resistance to Cry2Ab. Here we show that ABC-A3 is essential to Cry2A toxicity in fall armyworm (FAW), *Spodoptera frugiperda,* through CRISPR/Cas-mediated gene editing. We introduced edits in different regions of the ABC-A3 gene that are predicted to result in premature translation termination which resulted in survival on high doses (~LC_{95}) of Cry2Ab-like protein in artificial diet bioassays. We will report molecular characterization of the ABC-A3 knockout insects compared to a susceptible laboratory colony.

Contributed paper Wednesday 11.15 **97**

Investigation into specificity determinants of Vip3Bc1

Marc Zack[1], Megan Sopko[1], Scott Bevan[1], Ted Letherer[1], Sek Yee Tan[1], Yolanda Bel[2], Baltasar Escriche[2], and Ken Narva[1]
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Vip3 vegetative insecticidal proteins are soluble members of the *Bacillus thuringiensis* (Bt) family of insecticidal proteins proven to be active on lepidopteran insect pests. In general, it is accepted that Vip3 proteins represent an insecticidal mode of action different than that of the more-studied 3-domain crystal Bt family of toxins. Therefore, the Vip3 family has become a valuable tool for development of insect-resistant crops such as corn and cotton. To date, three subsets of the Vip3 family have been identified; Vip3A, Vip3B, and Vip3C. We have recently shown that Vip3Bc1, and new member of the Vip3B family, has an insecticidal spectrum unique from that of Vip3A proteins. Aside from a distinguishing and limited spectrum, Vip3Bc1 proteolytic processing is severely attenuated compared to Vip3Ab1. Lastly, our previous work indicates that the C-terminal region functions to direct activity towards target pests. Therefore, in the current work, we have used these attributes and inherent sequence divergence of Vip3Ab1 and Vip3Bc1 to further investigate the role of processing and midgut BBMV binding as determinants of Vip3Bc1 specificity.

Contributed paper Wednesday 11.30 **98**

Selection for UV-resistance in the Cryptophlebia leucotreta betabaculovirus for a more persistent biopesticide

Patrick Mwanza[1], Gill Dealtry[1], Michael Lee[2] and **Sean Moore**[3, 4]
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Formulations of the betabaculovirus, Cryptophlebia leucotreta granulovirus (CrleGV), have been used commercially for control of the false codling moth, *Thaumatotibia leucotreta*, on citrus in southern Africa since 2004. In order to provide farmers with improved clarity on frequency of application required, the rate of breakdown of the virus after application in the field, due to ultraviolet radiation, was determined. This was done through a combination of field trials and laboratory bioassays, which indicated that despite exposure to UV for several days, there was always some residual activity. It was postulated that this residual activity arose from the presence of UV-resistant CrleGV-SA in the virus population. The aim of this study was to isolate this UV resistant CrleGV-SA. To achieve this, samples of CrleGV-SA 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 LC_{50} values. After 24 h exposure, LC_{50} was 2.89 x 10^8 OBs /ml at cycle 1, 1.19x 10^8 OBs /ml at cycle 2 and 1.15 x 10^5 OBs /ml at cycle 3. LC_{50} values after 72 h exposure were 2.11 x 10^9OBs /ml at cycle 1, 1.5 x 10^9 OBs/ml at cycle 2 and 8.18 x 10^6 OBs/ml at cycle 3. The decrease in LC_{50} at cycle 3 may indicate successful isolation of resistant CrleGV-SA. The final two cycles must still be bioassayed. Transmission electron microscopy (TEM) studies being conducted concurrently showed that UV damaged and destroyed the nucleocapsid. TEM images of UV-irradiated CrleGV-SA also showed a deterioration in the OB, demonstrating that UV damage can be visually observed.

Contributed paper Wednesday 11.45 **99**

The potential use of a novel alphabaculovirus as a microbial control agent against three economically important tortricid pests

T.Marsberg[1,3], M.D. Jukes[2], M.P. Hill[1], C. Knox[2], S.D. Moore[1,3], B. Szewczyk[4], L. Rabalski[4] and J. A. Jehle5

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Cryptophlebia peltastica (Meyrick) (Lepidoptera: Tortricidae) is an economically damaging pest of litchis and macadamias in South Africa. A laboratory culture of *C. peltastica* was established and maintained at Rhodes University for the potential isolation of a baculovirus that could be used as a biopesticide against this pest. Larvae showing symptoms of viral infection were collected and examined for the presence of a baculovirus. A nucleopolyhedrovirus was initially identified by transmission electron microscopy. Further characterisation of this virus through OB sectioning and whole genome sequencing, revealed that this virus was a novel alphabaculovirus, which is now referred to as Cryptophlebia peltastica nucleopolyhedrovirus (CrpeNPV). Bioassays were conducted to determine the virulence of CrpeNPV against three economically important Tortricid pests, *C. peltastica, Thaumatotibia leucotreta* and *Cydia pomonella.* Bioassays for *C. pomonella* were completed on both CpGV resistant and non-resistant larvae. CrpeNPV was found to be more virulent against *T. leucotreta* and *C. pomonella* (CpGV resistant and non-resistant) than its homologous host. Currently, CrpeNPV is being tested in citrus orchards in South Africa with encouraging results for the control of *T. leucotreta*. Further research will focus on mass producing the virus and determining the efficacy in the field.

Contributing paper Wednesday `12.00 **100**

Developing RNA interference as a species-specific biological insecticide

Ernesto Soto[1], James Baum[2], Jodi Beattie[2], Stephen Beishir[2], Michelle Gasper[2], Steven Halls[2], Jennifer Howard[2], Joanna Pawlak[2], Tanusri Samanta[2], **Gary Ostroff**[1]

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Developing RNA interference as a species-specific, biological insecticide has many challenges: 1) identification of lethal insect gene targets, 2) cost-effective manufacturing process, 3) stably bind the RNAi mediator to a plant surface until ingested, 4) efficient release of the RNA payload in the midgut of the insect and 5) efficient RNA uptake into midgut epithelial cells. The outer cell wall structures of fungi have evolved to adhere and colonize plant surfaces through lectin-carbohydrate interactions. Further, many species of insects view fungi as food and are able to ingest 3-4 micron-sized yeast cell wall particles (YCWPs). We hypothesized that combining our expertise in packaging nucleic acids inside YCWPs together with Monsanto's expertise in insect genomics, physiology and insecticide screening offered the potential of addressing hurdles 3-5 above and test the practicality of using RNAi as a biological insecticide. We chose Colorado potato beetle (CPB), a model organism with commercial potential killed by free RNA in CPB in vitro feeding assays (Zhu et. al., 2011). We first screened which YCWP type best bound to plant surfaces, resisted wash off and CPB ingested. These experiments identified the importance of the outer YCWP mannoprotein coat for leaf retention and ingestion, and we chose yeast glucan-mannan-lipid particles (GMLP) for further work. Next, we evaluated two different GMLP RNA delivery approaches: 1) formation of soft RNA-polymer complexes or 2) binding RNA to hard cationic nanoparticles inside the GMLP hollow cavity. Both RNA delivery strategies were effective at encapsulating RNA (up to 90% encapsulation efficiency), and delivered RNA into the CPB digestive tract achieving up to 100% killing within 9-12 days. Comparing the efficacy of free RNA (Ec_{50} = 32 ng RNA/ml) to GMLP hard nanoplex formulated RNAs (Ec_{50} = 10 ng RNA/ml) to GMLP soft nanoplex formulated RNAs (Ec_{50} = 5 ng RNA/ml) demonstrated a 3-6 fold enhancement in median EC_{50} for GMLP formulated RNAs over free RNA. These results support the continued development

of GMLP formulated RNAs as a biological insecticide against CPD.

Contributed paper Wednesday 12.15 **101**

Formation and dispersion mechanisms of *Bacillus thuringiensis* biofilm and its potential biocontrol application

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Bacillus thuringiensis (Bt) produces insecticidal crystal proteins (ICPs) and exotoxins (α, β, and γ-exotoxins), toxic to agricultural and forestry pests, and harmless to the environment and non-target organisms. Since the Bt-derived ICPs are particularly susceptible to sunlight (ultraviolet ray, UV), Bt has a relatively short field duration. Bacterial biofilms (BBFs) are cell populations that grow on the surfaces of solid-liquid and liquid-gas, and are entrapped in the exopolysaccharide matrix. It can improve the UV protection ability of bacteria against environmental stress. Therefore, the research of Bt biofilm may provide a new choice for solving the above bottleneck problem. First, the BBFs and their applications are reviewed. Then the effects of biofilms on Bt biocontrol activity, the regulation mechanism of Bt biofilm formation and dispersion, and the identification methods of Bt biofilm related genes were introduced. The results showed that Bt biofilm can effectively improve its resistance against UV ray. And it is expected to extend the field duration of Bt preparations. The prospects for the research direction of BT biofilms are also discussed. It is believed that through the tireless efforts of Bt researchers, BBFs will play an important role in basic research and practical application of Bt in recent years.

POSTERS

WEDNESDAY 16 AUGUST 13.30-15.30 CONFERENCE FOYER

BACTERIA

Poster/Bacteria. Wednesday, 13.30. **BA-1**

A toxin-antitoxin system is essential for the stability of mosquitocidal plasmid pBsph of *Lysinibacillus sphaericus*

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Lysinibacillus sphaericus C3-41 carries a large low-copy-number plasmid pBsph, which encodes binary toxin proteins. Our previous study found that the transcriptional activator TubX plays an important role in the newly identified type Ⅲ TubRZC replication/partition system in pBsph, and that the constructed vector consisting of *tubRZC* and *tubX* is still not as stable as pBsph, indicating the presence of other maintenance module(s). In this study, a series of deletion and complementation for pBsph were performed, which found that the deletion of *orf9-orf10* resulted in a more obvious loss rate than other mutants, whereas the complementation of *orf9-orf10* restored the plasmid stability. Bioinformatics analysis showed that ORF9 contains a PIN domain of VapBC toxin-antitoxin (TA) system, whereas ORF10 share no significant sequence similarity to any of the characterized antitoxins in the database. Further studies revealed that the *orf9-orf10* is transcribed as an operon, and the overexpression of ORF9 repressed the growth of both *Escherichia coli* and *L. sphaericus*, which can be alleviated by overexpression of ORF10, confirming that ORF9 and ORF10 work as a toxin and antitoxin, respectively. In addition, the instability of pBsph in Δ*orf9-10* can be recovered by overexpression of TubRZ, and the recombinant vector containing *tubRZC*, *tubX* and *orf9-10* was more stable than the ones only containing *tubRZC* and either *tubX* or *orf9-10.* The data indicates that the complete plasmid maintenance system on pBsph is composed of *orf9-orf10* TA system and our previous identified *tubRZC* and *tubX*. The work makes a good complementary for the study on type III partition/segregation system, helps our standing for the plasmid stability and builds up a thesis basis for genetic engineering of *L. sphaericus*.

Poster/Bacteria. Wednesday, 13.30. **BA-2**

Binding studies, insect bioassays, and field trials on a modified Vip3C protein and other proteins active against *Spodoptera frugiperda* and *Helicoverpa armigera*

Theodore W. Kahn[1,†], Maissa Chakroun[2,†,i], Jayme Williams[1], Tom Walsh[3], Bill James[3], Jessica Monserrate[1] & **Juan Ferré[2]**
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A modified Vip3C protein has been developed that has an altered spectrum of activity, and shows good efficacy against *Spodoptera frugiperda* in insect bioassays and field trials. For the first time Vip3A and Vip3C proteins have been compared to Cry1 and Cry2 proteins in a complete set of experiments

from insect bioassays to competition binding assays to field trials, and the results of these complementary experiments are in agreement with each other. Binding assays with radiolabelled toxins and brush border membrane vesicles from *S. frugiperda* and *Helicoverpa armigera* show that the new Vip3C protein shares binding sites with Vip3A, and does not share sites with Cry1Fa or Cry2Ae. No other shared binding sites were found among these four toxins. In agreement with the resulting binding site model, *H. armigera* Vip3A-resistant insects were also cross-resistant to the new Vip3C protein. Furthermore, maize plants expressing the modified Vip3C protein, but not those expressing Cry1F protein, were protected against Cry1F-resistant *S. frugiperda* in field trials.

Poster/Bacteria. Wednesday, 13.30. **BA-3**

Characterization of a novel *Lysinibacillus sphaericus* myovirus vB_LspM-01 displaying pseudolysogeny

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Lysinibacillus sphaericus is one of the best commercialized bacterial pesticides used for mosquito control programs to reduce the mosquito-transmitting tropical diseases. Phages contribute to the genetic diversity and niche adaptation of bacteria, but their infection may bring huge economic damages during the industrial fermentation for those have good application values. The occurrence of phages in *L. sphaericus* remains poorly characterized. In this study, one phage specifically infecting *L. sphaericus*, named vB_LspM-01, was isolated from *L. sphaericus* C3-41. Morphology observation showed that vB_LspM-01 belonged to *Myoviridae* family with a spheroidal head and contractive tail. It was sensitive to 16 out of the 21 tested *L. sphaericus* isolates, but resistant to the other species isolates. Sequencing analysis revealed that vB_LspM-01 had a circular dsDNA genome with a size of 50,753 bp encoding 84 ORFs, which had no obvious similarity with that of its host C3-41. About 67.9% putative ORFs of vB_LspM-01 were predicted as hypothetical proteins with unknown function, of which 33.3% could not get a match at all within the public database by blast, indicating that vB_LspM-01 might be a novel unclassified viral species. The phylogenetic analysis based on terminase large subunit showed that the phage might use a *pac*-type DNA headful packaging mechanism to package the double-strand viral DNA into the prohead. The low prevalence and instability of vB_LspM-01 in different clones of C3-41 upon cell passage were observed, indicating that it was present as a pseudolysogeny state in the population. In addition, the phage endolysin was cloned and characterized, which displayed a broader antimicrobial spectrum than the phage. The work explored the hidden pseudolysogenic phage in *L. sphaericus*. As we know this is the first reported phage derived from *L. sphaericus*, which enriches the genetic pool of *L. sphaericus* and helps our understanding on the interaction and co-evolution between phage and host.

Poster/Bacteria. Wednesday, 13.30. **BA-4**

Comparison of two carriers to formulate baits based on *Yersinia entomophaga* to control the African black beetle (*Heteronychus arator*)

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A bait formulation is an active ingredient mixed with food or another attractive substance. The bait either attracts the pests or is placed where the pests will find it and insects are killed by eating the bait that contains the pesticide. One isolate of *Yersinia entomophaga* MH96*,* a gram-negative, rod-shaped, non-spore-forming bacterium isolated from diseased larvae of the New Zealand grass grub, *Costelytra giveni,* was formulated as baits using two natural carriers (only corn or a mixture of corn and wheat). Baits with initial bacteria loading of 10^6 and 10^7 CFU/g and moisture content 7±1% were placed on the surface of sterilized field-collected soil (22% moisture content) in the laboratory and bait samples were tested for moisture and bacteria loading after 24, 48, 96 and 144 hours. Moisture content and bacteria loading increased significantly in all treatments during the first 96 hours, reaching a maximum level of 20% and 10^{10} CFU/dry g respectively, then began to decrease after this time. Bacteria growth and water uptake were faster when the carrier was based on corn in comparison with the corn and wheat mixture. When baits were stored in vacuum-sealed aluminum bags, bacteria loading declined by less than one logarithmic unit after six months of storage at 4°C with no differences between carriers. Finally, baits were tested for palatability against black beetle adults (*Heteronychus arator*) under laboratory conditions. Dry and damp baits were given to black beetles that were allowed to feed for 4 days. More beetles fed on damp baits and when corn was used as principal carrier. Given the beetle's preference for corn and the capacity of this carrier to uptake water and promote bacteria growth, baits formulated with corn were selected to continue developing a biopesticide to control black beetle in New Zealand pastures.

Poster/Bacteria. Wednesday, 13.30. **BA-5-STU**

Diversity and distribution of *cry* genes in *Bacillus* spp. strains using a universal PCR primer system and hidden Markov model profiles from the C-terminal end of Cry proteins.

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Bacillus thuringiensis (Bt) is the main microorganism used for biological control of insects, it has been used in the development of many products for the control of pests, however there are some limitations that have prevented Bt from increasing its success, as its competitiveness against synthetic insecticides is still low. These limitations can be surmountable through the search for new toxins with higher insecticidal activities, the improvement of the known toxins, the optimization its industrial production, the efficiency of field application, and the expansion of toxins used in transgenic plants. Interestingly, there are reports of *cry* genes in bacteria other than Bt, which may increase the potential use of these toxins. This work focuses on the identification of new *cry* genes in bacteria other than Bt through the use of universal primers designed from the blocks conserved in the family of *cry* genes. Some 223 strains were analyzed with the previously standardized three sets of primers, of which 43 amplicons were obtained, and 12 were cloned and sequenced. Six of these sequenced amplicons tested positive for Cry-like proteins, which were analyzed and its complete sequence was obtained. Additionally, *cry* genes were searched in the genomes reported in databases by developing a profile of hidden Markov model (HMM profile) designed from the C-terminal end of known Cry proteins. The model was able to identify complete Cry proteins containing a C-terminal end. A total of 857 genomes of *Bacillus* spp. were analyzed and 174 putative Cry proteins were identified in Bt and other *Bacillus* spp. genomes.

Poster/Bacteria. Wednesday, 13.30. **BA-6**

Enriching and mining soil and grain metagenomes for novel insecticidal proteins

Irina Shilova[1], Amy Jo Johnson[1], Alex Gulevich[1], Ryan Dowdy[1], Sunit Jain[1], Paul Loriaux[1], Ryan J Williams[2], Ian W Davis[2], Jeff A Haas[2], Shoko Iwai[1], Prasanna Ramachandran[1], Erica Rutherford[1], Kim M Wegener[2], Thomas Weinmaier[1], Yonggan Wu[1], James Baum[2], Todd Z DeSantis[1], Karim Dabbagh[1], **Kristen Bennett**[1]

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Insecticidal microbes and their products have been successfully used as effective and environmentally safe solutions against agricultural pests for more than half a century. However, identifying new insecticidal proteins that can overcome resistance is important to the long-term durability of this cropping system. Conventional approaches to discover novel insecticidal toxins are largely based on spore-selection and strain isolation techniques. Metagenomics allows tapping into the world of uncultured microbes. Soil is an extremely rich source of novel proteins, but high microbial diversity and relatively low representation of microbes that produce insecticidal proteins in soil are prohibitive for assembling genes for insecticidal proteins. To overcome this limitation, we have developed enrichment techniques and metagenomics pipelines coupled with metatranscriptomics approaches to identify novel insecticidal toxins from soil and other environmental samples such as grain dust samples. We used a mix of organic nitrogen and carbon nutrients to facilitate the growth of insecticidal bacteria. The resulting microbial community may have reduced diversity and increased representation of the desired taxa, which can more successfully be used in 'omics-based approaches. We will present the results of mining metatranscriptomes and metagenomes from soil and grain microbial communities in response to enrichments using the Second Genome discovery platform to specifically identify insect toxin genes.

Poster/Bacteria. Wednesday, 13.30. **BA-7-STU**

Development of a Bacterial Pesticidal Protein Resource Center

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To date more than 700 *cry* gene sequences that code for crystal (Cry) proteins have been formally identified. Most of these have been isolated from strains of *Bacillus thuringiensis* although an increasing number are being found in other bacterial species. The primary goals of our project are to establish and build an online pesticidal database and to review the existing Bt toxin nomenclature to better reflect the greater diversity that now exists among these pesticidal proteins. A number of tools have been considered to facilitate the development of an automated classification system. These include the use of Pfam (http://pfam.xfam.org/) to group the proteins into distinct structural groups - since all but a handful of the current proteins match an existing Pfam definition. To further characterize the proteins,

various alignment methods (Clustal Omega, Muscle, and T-coffee) have been evaluated alongside a non-alignment based method (MASH). In addition to using multiple sequence alignments we have also evaluated the potential of using just pairwise alignments to inform a classification system. This poster will present the results of these analyses and describe the approach that will be used for the revised classification system. The Bt toxin nomenclature exists as a simple online list at present and so work is underway to create a functional online database that will facilitate a number of further analyses and metadata presentation. We will present details of how this database will be constructed.

BENEFICIAL INVERTEBRATES

Poster/Dis. Ben. Wednesday, 13.30. **DB-1-STU**

Artificial insemination techniques and sexually transmissible diseases in honey bees (*Apis mellifera*)

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Advanced reproductive technologies including artificial insemination (AI) are routinely used in mammalian species to facilitate genetic selection and improve breeding outcomes. AI is also used in honey bees (*Apis mellifera*). Many techniques used in queen bee insemination were established in the mid-twentieth century, with limited technical advances since the 1970s. Many routine procedures have not been empirically tested to determine if they are necessary or desirable. My PhD project will critically evaluate common AI procedures. For example, carbon dioxide is used to provide narcosis and induce egg laying in artificially inseminated queens. However, the optimal timing, duration, and number of narcoses is not well understood. Queens are typically inseminated between 5 and 10 days of age, but is there an optimum? Should queens receive their second narcosis before or after insemination? A second aim of my PhD is to critically assess the biosecurity risks associated with importation of bee semen. Deformed Wing Virus (DWV) has been shown to be transmissible in honey bee semen, and can establish vertical transmission from affected queen to her progeny. *Nosema* spp. microsporidia have been identified in semen, and have been demonstrated to be transmissible by this route in artificially inseminated queens. *Nosema* and DWV are unlikely to be the only sexually transmissible diseases present in honey bees, so I will conduct a thorough survey of ejaculates to investigate the possibility of any other sexually transmitted diseases in honey bees.

Poster/Dis. Ben. Wednesday, 13.30. **DB-2-STU**

Characterization of the major sensory organ and Ionotropic receptors of *Tropilaelaps mercedesae*

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Tropilaelaps mercedesae is major threat to honey bee health and colony in addition to *Varroa destructor* in Asia. *T. mercedesae* not only feeds on hemolymph of honey bee but also transmits deformed wing virus (DWV) as *V. destructor* and builds up relatively higher population levels within colonies by producing a higher number of offspring. *T. mercedesae* is currently restricted to Asia but it has the potential to spread and establish across the globe due to the global trade of honey bees. However, we still don't understand the general biology of *T. mercedesae* such as the sensory system, development, sex determination/differentiation, reproduction, and the capability to acquire miticide resistance. To understand how *T. mercedesae* finds the fifth instar honey bee larva and responds to other environmental cues, we characterized the structure/morphology of major sensory organ specifically present in the foreleg by SEM and compared it with those of other mites and ticks. Consistent with the presence of major sensory organs, we found that genes highly expressed in the forelegs compared to other legs of *T. mercedesae* are enriched with many GO terms associated with ion channel and cilium by RNA-seq analysis. Particularly, ionotropic receptors (IRs) represented the major ion channels highly expressed in the forelegs. IRs are protostome-specific ion channels originated from glutamate receptors and play various roles for sensory perception. We found that *T. mercedesae* expresses the orthologs of *Drosophila melanogaster* IR25a and IR93a in the forelegs and the fruit fly genes were previously shown to be involved in the thermosensation and hygrosensation. We therefore tested whether the behavioral defects of *D. melanogaster* IR25a and IR93a mutants can be rescued by introducing the *T. mercedesae* orthologs (TmIR25a and TmIR93a). We have found that TmIR25a and TmIR93a rescue the thermotactic defects of *D. melanogaster* IR25a and IR93a mutants, demonstrating that the functions of IR25a and IR93a in thermosensation are well conserved. These results suggest that the sensory organs in the forelegs of *T. mercedesae* contain two divergent family of proteins, TmIR25a, TmIR93a, and previously characterized TRPA1 channel (TmTRPA1) to respond to temperature changes in the environment.

Poster/Dis. Ben. Wednesday, 13.30. **DB-3**

Establishment of *Bacillus thuringiensis* based exogenous double-stranded RNA production platform

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RNA interference (RNAi) has been considered as an alternative strategy to control agricultural pests whereby double-stranded RNA (dsRNA) triggers a potent and specific inhibition of its homologous mRNA. Since small dsRNAs are required for various RNAi applications, there is a need for cost-effective methods for producing large quantities of high-quality dsRNA. To produce exogenous dsRNA through simple and cost-effective methods, *Bacillus thuringiensis* (Bt) based dsRNA production platform was established. For this, Bt shuttle vector, pHT1K-vp1, which transcribes sense and anti-sense *vp1* gene of Sacbrood virus (SBV) under the control of sporulation-dependent *cyt1*Aa promoter with STAB-SD sequence was constructed and transformed into Bt 4Q7 strain. Transcription of the *vp1* gene was analysed using qPCR and Northern blot analysis. In addition, the dsRNA against *vp1* gene produced from the Bt successfully suppressed the replication of SBV. These results suggested that the Bt potentially exploited as a new platform for dsRNA production.

Poster/Dis. Ben. Wednesday, 13.30. **DB 4**

Trypsin-mediated maturation of a novel antimicrobial peptide is important to resist bacterial infection in red swamp crayfish *Procambarus clarkii*

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Antimicrobial peptides (AMPs) are a key family of effectors involved in host defence of both vertebrates and invertebrates. By analysing the transcriptome data of healthy and *Aeromonas hydrophila*-infected red swamp crayfish *Procambarus clarkii*, a novel AMP, designated PcnAMP, was found significantly inducible by *A. hydrophila* infection. Two native forms of PcnAMP existed *in vivo*, with the long form as the full length PcnAMP, and the short form as the N-terminus of PcnAMP of 39 residues. The short form was found cleaved from full length protein at a trypsin-cleavage site, and the endogenous trypsins were responsible for the proteolytic processing. *A. hydrophila* infection could induce the trypsin-mediated maturation of PcnAMP. The short form adopted a well-defined amphipathic alpha-helix structure, and showed some similarity with the frog antimicrobial peptide uperin. Both the long form and short form exhibited antimicrobial activity, and the activity of the short form was much higher than that of the long form. PcnAMP showed a strong protective role against *A. hydrophila in vivo*. The identification and characterization of a novel AMP provided new potential of development of therapeutic agent for disease control in aquaculture.

Poster/Dis. Ben. Wednesday, 13.30. **DB-5-STU**

Impacts of change of environment on the composition of microbiota in Australian stingless bees, *Tetragonula carbonaria*.

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The composition of the bee microbiome has been shown to have remained stable across millennia. However, over a shorter timeframe, variations within and between the microbiota of colonies may illuminate key questions on the environmental versus inherited origins of the microbiome and, potentially, of interactions between symbionts.

We used next generation sequencing to compare the microbiome of Australian stingless bees (*Tetragonula carbonaria* Smith) from pairs of related hives that had been separated across locations, preventing overlap in foraging. DNA was extracted from 6 whole bees from each colony and the V3-V4 region of the 16S rRNA gene 'barcode' was amplified and sequenced using the Illumina MiSeq. Reads were analysed using new bioinformatics tools to identify Exact Sequence Variants and to determine diversity within our sequencing data at the highest resolution possible. Database-independent analysis was used to extend the exploration beyond taxonomic assignment, and statistical measures were used to identify highly similar sequences that have dissimilar patterns of occurrence.

Patterns of co-occurrence and absence of variants were used to describe diversity and explore potential cooperation and competition between symbionts. Microsatellite analysis of the individual bees will be used to determine the effects of kinship and environment on bee microbiota.

Poster/Dis. Ben. Wednesday, 13.30. **DB-6-STU**

Stress and the bee: the impacts of hive design and management practice on honey bees, *Apis melifera* L.

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Hive design for honey bees (*Apis melifera* L.) has changed little since Langstroth introduced his Patent Hive in 1854, but the use of hives in pollination and industrial production has changed the use of hives. In particular, hive inspection and honey collection may introduce stress and spread pathogens that could be mitigated by changes in practice and hive design. Colonies of the honey bee maintain a mean brood nest temperature within a narrow range of 34.5-35.5°C, which is critical for brood development and nectar evaporation, and invest significant energy to maintain these conditions. Honey harvesting processes and removal of honey-filled comb may have a significant impact on brood nest temperature that would require additional investment of energy by the bees in order to restore thermal equilibrium and diverting workers away from other important tasks. As part of a wider study on the impacts of apicultural practice on stress and bee health, we quantified the thermal energy loss resulting from honey harvesting and common inspection practices in conventional Langstroth hives.

The results show significant energy expenditure by bees is required to rectify the hive micro-environment after honey harvesting and colony inspection, and highlight the importance of thermal mass in maintaining the hive environment. Identification of the impacts of current practice and hive design allow for improvement in the design of bee hives to reduce stress on the bee colony, and contributing to increased colony function.

Poster/Dis. Ben. Wednesday, 13.30. **DB-7-STU**

A new record of chalkbrood, *Ascosphaera solina*, isolated from and Australian native bee (*Amegilla cingulate*) of the Family *Apidae*

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Chalkbrood, an infection of bee larvae caused by fungi of the genus *Ascosphaera*, is widespread in honey bees *Apis melifera* L. and bumble bees (both family *Apidae*). It is also found in American and European species of solitary bees of the family Megachilidae. Chalkbrood is common in European honey bees in Australia, but infection by *Ascosphaera* spp. in Australian native bees is not widely reported. However, the few reported incidences identified several new species from Australia from cadavers of the families Megachilidae and Colletidae. Infection in native *Apidae* has not been reported.

As part of a larger study on incidence and transmission of diseases to Australian native *Apidae* we examined nest blocks used by multiple solitary native bee species. A fungus was isolated from an adult cadavers of the Australian blue banded bee, *Amegilla cingulate* (Fabricius) (Apidae) and characterised. Sanger sequencing of amplicons of the ITS region identified the fungus as *Ascosphaera solina*, which has only been reported once, also in Australia, from an unknown mummified larvae of the family Colletidae. This is the first time that an *Ascosphaera* sp. has been identified in an Australia bee of the family Apidae. We discuss this in the context of disease transmission within Australian native bees.

Poster/Dis. Ben. Wednesday, 13.30. **DB-8**

Yeasts associated with nests of Australian stingless bees (*Meliponini*)

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Australian eusocial stingless bees of the tribe Meliponini raise young on honey and pollen stored in pots made of resin-beeswax. These provisions harbour a diverse microbiome, including bacteria and fungi, that can contribute to improved nutritional value and preservation and thus to the overall health of the bee colony. Chemical analyses of Australian pot-pollen have suggested that some bioactive volatiles might originate from microbial fermentation. The gut microbiota of Australian Meliponini has been studied, but the microbiota associated with their nests remains unexplored.

In this study, fungi and yeasts were isolated using selective high-osmolarity media and antibacterial agents from pot-pollen, pot-honey, brood cell walls ('cerumen') and food provisions from nine colonies of three Australian Meliponini species (*Tetragonula carbonaria*, *T. hockingsi* and *Austroplebeia australis*) located at the same site in South East Queensland. Bees from the 9 colonies were assumed to be foraging within the same radius. Fungi and yeasts were characterised by microscopy, sequencing of ITS and LSU 'barcodes' and subsequent BLAST search against GenBank, phylogenetic tree construction and amplicon concatenation. The relative abundance of bacteria in pot-pollen was estimated from 16S

'barcodes' by PCR amplification and Next Generation Sequencing using the MiSeq platform. Organisms identified include Ascomycota, Basidiomycota, Firmicutes and Proteobacteria previously unreported in nest materials of Australian Meliponini. The distribution of the microorganisms across the three bee species and their nest materials are described. Further analysis of yeast-derived volatiles is on-going as part of a larger project on the relationships between Australian Meliponini bees and yeast symbionts.

Poster/Dis. Ben. Wednesday, 13.30. **DB-9-STU**

Characterization of the cross-interactions between Deformed Wing Virus (DWV), honey bee and the ectoparasitic mite, *Tropilaelaps mercedesae*

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Recently, honey bee colony losses especially during winter have been reported to be associated with the presence of both ectoparasitic mites and Deformed Wing Virus (DWV). In addition to *Varroa destructor*, *Tropilaelaps mercedesae* is also prevalent in Asia. *T. mercedesae* potentially imposes more severe impact on honey bee colony with no phoretic period and better fecundity than *V. destructor*. However, the effects of *T. mercedesae*'s infestation on honey bee colony and health, particularly DWV transmission have not been fully investigated. We therefore tested 1) the effects of *T. mercedesae*'s infestation on DWV infection in honey bee by characterizing individual pairs of honey bee pupa and the infesting mite, and 2) the relationship between DWV and *T. mercedesae.* We found that *T. mercedesae*'s infestation increases DWV copy number in the honey bee pupae; however, the variants present in the pair of pupa and mite do not always match. There was a positive correlation of DWV copy number between pupae and the infesting mites, and the pairs of pupa and mite were grouped to two clusters with either high or low copy number of DWV. These results suggest that *T. mercedesae* functions as a vector transmitting DWV to honey bee pupae and are consistent with a previously proposed hypothesis that DWV suppresses the honey bee immune system when DWV copy number reaches a specific threshold. To give insight into the relationship between DWV and *T. mercedesae*, we analysed the transcriptomes of *T. mercedesae* with or without high copy number of DWV by RNA-seq. We found that vitellogenin, cuticle proteins, and serine protease inhibitors are down-regulated in *T. mercedesae* with high copy number of DWV. Consistent with the down-regulation of vitellogenin, we also found that the reproductive capability of *T. mercedesae* is negatively correlated with the copy number of DWV. These results may suggest that DWV decreases the fitness of *T. mercedesae*. To further explore the effects of DWV infection on *T. mercedesae*, we have generated several antibodies against VP1 of DWV including the P-domain which is considered to bind the cell surface receptor. Characterization of these antibodies will also be presented.

FUNGI

Poster/Fungi. Wednesday, 13.30. **FU-1-STU**

The lethality of *Beauveria bassiana s.l.* secondary metabolites on *Anopheles stephensi*

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Entomopathogenic fungi (EPF) have an insecticidal effect to a broad range species of insect hosts. Insecticidal effects of EPF are mainly driven by two factors: (1) disruption of host organs and tissues and, (2) production of secondary metabolites in host hemocoel. In our previous study, the high virulence strain *Beauveria bassiana s.l.* 60-2 induced early death of *Anopheles stephensi* by invading the head and brain. However, in some cases, we detected dead mosquitoes without fungal invasion to brain, and it shows the possibility of secondary metabolite effect. It is known that changing nutritional condition or fungal strain led to change the lethality of EPF secondary metabolites. The present study aims to evaluate the effect of secondary metabolites of *B. bassiana s.l.* on survival of *A. stephensi* through micro-injection of fungal culture filtrate, and evaluate the difference of lethality and/or virulence on each nutritional conditions or fungal strains. Initially, *B. bassiana s.l.* 60-2 culture filtrate was produced by using five different types of liquid medium. *A. stephensi* were injected with 65 nL of culture filtrate from each medium and evaluated survival rate. As a result, *B. bassiana s.l.* 60-2 culture filtrate from Czapek-Dox broth with yeast extracts, sabouraud broth, and sabouraud broth with yeast extracts showed high lethality to *A. stephensi*. Thus, there was possibility that nitrogen is the trigger of high lethality of *B. bassiana s.l.* 60-2 culture filtrate. Furthermore,

lethality of *B. bassiana s.l.* 60-2 culture filtrate was lost by heat treatment. It shows that part of factors for lethality in culture filtrate are proteins. Finally, fungal culture filtrate from Czapek-Dox broth with yeast extract and conidial suspension of *B. bassiana* 2112 which indicated low virulence in percutaneous infection showed high virulence to *A. stephensi* through micro-injection. This suggest that, *B. bassiana* 2112 has low ability to proliferate the fungal propagules and/or produce secondary metabolites after penetrating host integuments. And, the difference of immune responses in *A. stephensi* can be a cause for different virulence between *B. bassiana* 2112 and *B. bassiana s.l.* 60-2 in percutaneous infection.

Poster/Fungi. Wednesday, 13.30. **FU-2**

Biology and control with the fungus *Metarhizium anisopliae* of *Demotispa neivai* (Coleoptera: Chrysomelidae) a pest of oil palm in Colombia

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The fruits scraper of oil palm, *Demotispa neivai,* causes significant palm oil loss due to the damage to the epidermis when feeding on oil palm fruit. Initially, the life cycle was studied under laboratory conditions (28.8°C ± 1.2°C and 76.8 ± 6.3% RH). *D. neivai* life cycle was as follows: the egg stage lasted 7.1 ± 1.2 days, larvae 21.9 ± 2.0 days, passing through five instars, pupal stage 19.6 ± 3.0 days and adult survival lasted 268.9 ± 53.1 days. The insect's life cycle was thus completed in 309.1 ± 54.3 days. Their most important mortality factor found was the fungus *Metarhizium anisopliae* CPMa1502 strain. To evaluate this strain, pathogenic tests were performed under laboratory conditions, together with another strain of *M. anisopliae* (CeMa9236) against larvae and adults of *D. neivai*. Pathogenicity tests were made using 1 x 10^7 conidia/ml. Both strains of *M. anisopliae* were pathogenic. The virulence test was done under oil palm plantation infected with *D. neivai* populations using a dosage of 1 x 10^{13} conidia/ha. The CPMa1502 strain caused the highest larval mortality (87.7%), which was significantly different ($p<0.0001$) from strain CeMa9236. Then dosages (5 x 10^{12}, 7.5 x 10^{12} and 1 x 10^{13} conidia/ha) were tested using only strain CPMa1502, however, no significant differences in control were found among these dosages. Field evaluation in commercial plantation conditions was carried out with CPMa1502 at 5 x 10^{12} conidia/ha dosage in a plot with 511 oil palms. Larval mortality in the field 15 days after spraying was 62% and 30 days after spraying was 80%. The *M. anisopliae* CPMa1502 strain used at 5 x 10^{12} conidia/ha dosage was very effective to control of *D. neivai* in oil palm plantations.

Poster/Fungi. Wednesday, 13.30. **FU-3**

Diversity of entomopathogenic fungi from Kintrishi National Area forest ecosystem of Georgia

Medea Burjanadze[1], Stefan Jaronski[2], Ketevan Koridze[1], Archil Supatashvili[1]

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The Kintrishi Protected Areas incorporate the Kintrishi Nature Reserve (KNR) located between the Black Sea and the mountains of Adjara-Imereti. The undisturbed, protected territory with a unique micro-climate is well-known for a biodiversity of Caucasian and Colchis endemic and relict species. However studies on the occurrence and distribution of EPF in its soils are still lacking. We studied the diversity of entomopathogenic fungi in the KNR forest ecosystem by taking soil samples at 10 points through the forest in 2017. The distances between sampling points varied ranging from 1-2 km apart, and 500-1300 meters of altitude. At one site three soil samples were taken 25-100 m apart. In total, 30 soil samples were collected from this area. Entomopathogenic fungi were isolated from soil by (a) direct isolation from soil suspensions cultivated on PDAY-dodine media and (b) Galleria baiting using *Tenebrio molitor.* For further isolation entomopathogenic fungi different artificial medium SDAY and PDA were used. Identification of EPF was based on macro and micro characteristics following standard taxonomic keys and classification. The following entomopathogenic fungal taxa were found: *Beauveria bassiana* spp. (56% prevalence), *Metarhizium* spp. (12%), *Isaria* spp. (23%), *Isaria fumosorosea, Paecilomyces* sp., and *Fusarium* sp. (3.5%) from a total of 91 indigenous isolates.

Poster/Fungi. Wednesday, 13.30. **FU-4**

First records of *Beauveria* sp. and *Isaria* sp. occurrences in the invasive Brown Marmorated Stink Bug, *Halyomorpha halys* in Republic of Georgia

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The Brown Marmorated Stink Bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) (BMSB), is

an exotic invasive insect for Georgia, one that has spread extensively and established in the Black Sea regions of Georgia. At present BMSB is very active and undergoing considerable increase in large tracts of rural and urban West Georgia. Georgia is the third largest hazelnut-producing country worldwide, after Turkey and Italy. Following its first detection, BMSB has become a key pest in many crops, but especially in hazelnut orchards, and has caused considerable economic losses in recent years. The BMSB is so successful because of a lack of specific natural enemies, high fecundity, wide host range, resistance to cold weather, effective overwintering strategies, and is responding to global warming by increased survival. Our study aimed to explore the natural distribution of entomopathogens in Georgian BMSB populations. During 2017-2018 overwintering adults from different regions (Samegrelo, Guria, Imereti) of West Georgia were collected. More than 350 insects were dissected and assessed for infections. In March 2018 mycoses were observed among the collected insects. The fungi were isolated onto different agar media. Both *Beauveria* sp. *Isaria* sp*., Fusarium* sp. were identified.

Poster/Fungi. Wednesday, 13.30. **FU-5-STU**

Potential of *Metarhizium* strains isolated in New Zealand against the grass grub (*Costelytra giveni*)

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The grass grub, *Costelytra giveni* (Coleoptera: Scarabaeidae), an endemic pest of improved pasture in New Zealand, is a damaging root herbivore which lives in soil for most of its lifecycle. The pest is resistant to most microbes, but larvae are susceptible to some strains of the fungi belonging to the genera *Beauveria* and *Metarhizium*. However, fungal diseases are rarely sufficient to control the pest naturally. Around 5% of field-collected larvae were infected with *Metarhizium* spp., while a further 29% were dead due to undetermined causes. Strains were recovered from field collected grass grubs and the grass caterpillars, *Wiseana* spp. (Lepidoptera Hepialidae) and identified. Each recovered strain and those from existing collections were compared for virulence to grass grub larvae. Fourteen *Metarhizium* strains were isolated and identified, with two *M. novozealandicum* strains having higher virulence compared to other strains including *M. anisopliae, M. frigidum, M. pemphigi, M. brunneum, M. robertsii* and *M. guizhouense* after 28 days on second and third larval instars.

Poster/Fungi. Wednesday, 13.30. **FU-6**

Fungi *Metarhizium* spp. from Russia and neighboring territories: ecological preferences and activity against Colorado potato beetle larvae

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Thirty-five isolates of *Metarhizium* spp. from Russia and neighboring territories were genotyped using the 5′ EF-1α gene sequence analysis. Four species were identified, with *M. robertsii* and *M. brunneum* being the most frequent, and *M. anisopliae* and *M. pemphigum* being sporadic. *M. robertsii* was predominantly isolated from sun-opened habitats, but *M. brunneum* was isolated from forests. Radial growth studies in the temperature range of 10–40 °C revealed that growth at the high temperatures (35–37.5 °C) was inherent for *M. robertsii* isolates, but not for *M. brunneum* isolates. In contrast, *M. brunneum* isolates were more active at the cold temperatures (10 °C) compared to *M. robertsii*. Virulence was evaluated against the larvae of the Colorado potato beetle, *Leptinotarsa decemlineata* Say, under two regimes: humid (21 °C, 80% relative humidity (RH)) and arid (31 °C, 55% RH). *M. brunneum* isolates were less virulent compared to *M. robertsii* under the both regimes. *M. robertsii* activity did not differ under the two regimes, but *M. brunneum* was less virulent under the arid regime compared to the humid one. *M. pemphigium* was least virulent toward the beetle and was unable to colonize the hemocoel. A field experiment under the natural conditions (steppe zone of Western Siberia) with daily ranges of 10–43 °C and 13–98% RH showed that *M. robertsii* was significantly more active than *M. brunneum* against Colorado potato beetle larvae. The study was supported by grant of Russian Federation President (МК-6456.2018.11), Russian Foundation for Basic Research (project № 16-54-53033) and Russian Science Foundation (grant № 15-14-10014).

Poster/Fungi. Wednesday, 13.30. **FU-7**

Successful field trial of *Metarhizium* (Hypocreales: Clavicipitacea) based mycoinsecticide for *Musca domestica* (Diptera: Muscidae) control in Australian cattle feedlots

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House flies, *Musca domestica* L. (Diptera: Muscidae), are cosmopolitan pests of importance to public health and agriculture, especially intensive livestock systems including cattle feedlots. Uncontrolled fly populations are a significant nuisance and pose serious health and welfare implications for livestock, farm workers and surrounding communities through their capacity to transmit a variety of pathogens. Current house fly control practices incorporate a number of integrated pest management strategies including feedlot design, manure management and the release of parasitoid wasps, but is also heavily reliant on chemical insecticide applications. Excessive use of these insecticides has led to the development of resistance in *M. domestica* with some populations becoming resistant to almost all classes of insecticide used against them. Additionally, increased public health awareness regarding extended occupational exposure to insecticides has led to a need for safer alternatives for house fly management. Mycoinsecticide formulations based on entomopathogenic fungi offer one alternative to conventional chemical insecticides. Here, we develop and assess the efficacy of an ultra-low volume (ULV) mycoinsecticide formulation based on *Metarhizium anisopliae* in a field trial at commercial feedlots. Four feedlots were used for the field trial. Fly population numbers were monitored across all feedlots weekly and spraying of the mycoinsecticide was conducted fortnightly on two of the feedlots. The formulation was applied at a dose rate of approximately 1×10^{13} condia/ha. Application of the formulation significantly reduced field populations of *M. domestica* and the infection rate of *M. anisopliae* in sampled flies was high. The spray regime also significantly increased the background levels of *M. anisopliae* for up to two weeks post-spraying. The mycoinsecticide developed in this project could provide an important element in integrated fly management programs for cattle feedlots and other intensive livestock systems.

Poster/Fungi. Wednesday, 13.30. **FU-8**

New mycopesticides for lesser mealworm (*Alphitobius diaperinus*) control in poultry houses

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House flies, *Musca domestica* L. (Diptera: Muscidae), are cosmopolitan pests of importance to public health and agriculture, especially intensive livestock systems including cattle feedlots. Uncontrolled fly populations are a significant nuisance and pose serious health and welfare implications for livestock, farm workers and surrounding communities through their capacity to transmit a variety of pathogens. Current house fly control practices incorporate a number of integrated pest management strategies including feedlot design, manure management and the release of parasitoid wasps, but is also heavily reliant on chemical insecticide applications. Excessive use of these insecticides has led to the development of resistance in *M. domestica* with some populations becoming resistant to almost all classes of insecticide used against them. Additionally, increased public health awareness regarding extended occupational exposure to insecticides has led to a need for safer alternatives for house fly management. Mycoinsecticide formulations based on entomopathogenic fungi offer one alternative to conventional chemical insecticides. Here, we develop and assess the efficacy of an ultra-low volume (ULV) mycoinsecticide formulation based on *Metarhizium anisopliae* in a field trial at commercial feedlots. Four feedlots were used for the field trial. Fly population numbers were monitored across all feedlots weekly and spraying of the mycoinsecticide was conducted fortnightly on two of the feedlots. The formulation was applied at a dose rate of approximately 1×10^{13} condia/ha. Application of the formulation significantly reduced field populations of *M. domestica* and the infection rate of *M. anisopliae* in sampled flies was high. The spray regime also significantly increased the background levels of *M. anisopliae* for up to two weeks post-spraying. The mycoinsecticide developed in this project could provide an important element in integrated fly management programs for cattle feedlots and other intensive livestock systems.

Poster/Fungi. Wednesday, 13.30. **FU-9**

Influence of the plant hormone strigolactone on conidium germination and colonisation of plant roots by *Metarhizium anisopliae*

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Hypocrealean fungi of the genus *Metarhizium* are known rhizospheric-competent symbionts that support increased plant growth and development. However, the establishment of inocula in the rhizosphere is typically low. We investigated the role of plant: fungal signals and specifically of the plant hormone strigolactone in colonisation of pea plant roots by *M. anisopliae.* Germination of conidiospores in root exudate and colonisation of the root by *M. anisopliae* were estimated on wild variety pea (*Pisum sativum* L. cv Torsdag) and the mutants, rms 5-3: strigolactone-deficient and rms 4-1: strigolactone-overproducing mutants. Condiospore germination was significantly lower in

root exudates from the strigolactone-deficient pea (rms 5-3) than in exudates from wild and strigolactone -overproducing (rms 4-1) mutant plants. Colonisation of root segments was higher in wild and strigolactone -overproducing (rms 4-1) mutant plants than the strigolactone-deficient (rms 5-3) plants. This is the first report indicating that plant strigolactone increases both conidiospore germination and colonisation of the root by species of *Metarhizium*.

Poster/Fungi. Wednesday, 13.30. **FU-10**

Effects of entomopathogenic fungi and a commercial bioinsecticide on brown marmorated stink bug, *Halyomorpha halys* (Stål) (Heteroptera: Pentatomidae) under laboratory conditions

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The brown marmorated stink bug, *Halyomorpha halys* (Stål) (BMSB) is an exotic invasive insect in Georgia, originating in East Asia. It is a highly polyphagous pest, feeding on more than 300 plant species. BMSB was accidentally introduced into Western Georgia in 2015. In 2016, *H. halys* populations increased and high damage levels were observed in fruits, vegetables, maize, and hazelnuts, the most important crop of the region.

We performed laboratory tests of the biological activity of entomopathogenic fungi: *Isaria fumosorosea* (Ascomycota: Cordycipitaceae) isolated from *H. cunea* in Georgia (ARSEF 10244), isolate ARSEF 1569, a mixed suspension of the isolates, and the commercial biopesticides Nostalgist®, containing *Beauveria bassiana* against adults of *H. halys.* Adults of *H. halys* were collected in the Samegrelo region from trees of *Ficus* sp., transferred to the laboratory and starved for 24 hours. A conidial suspension was obtained by washing down the fungal rearing tubes with sterilized water. A single concentration (5 x 10^8 conidia/ml^{-1}) of entomopathogenic fungi and the recommended dose of the bioinsecticide were used in the experiments. Adults were sprayed individually with 1 mL of treatment suspension. Control adults were sprayed with water. Carrots, green beans, and potatoes were offered as food source and exchanged every 2nd day. The number of dead insects were determined at 3, 5, 7, and 9th days after application. Dead individuals were removed and individually placed in sterile Petri dishes on filter paper, moistened with distilled water and the sporulation of fungi was recorded. Efficacy, corrected with mortality in the control treatment, was calculated according to Schneider-Orelli's formula. The isolate ARSEF 10244, isolate ARSEF 1569, mixed suspension of both isolate and biopesticide Nostalgist® caused mortality of rates of 16.6%, 25%, 33.3% and 8.3%, resp., at day 3 post treatment. Mortality of adults increased to 45.4 %, 63.5%, 72.7% and 36.3% at day 9 post treatment with the resp. treatment. Both entomopathogenic fungal isolates were more effective against adults of BMSB than the bioinsecticide Nostalgist®, however highest mortality rates (72.7%) were achieved with the mixed suspension of both isolates.

MICROSPORIDIA

Poster/Microsp. Wednesday, 13.30. **MI-1-STU**

Molecular evidence of multiple microsporidian co-infections in mosquitos

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Introduction of molecular techniques based on DNA sequencing made great progress in microsporidia detection. However, most of the techniques relay on semi- or fully-specific primers and in consequence may lead to some species being overlooked, especially those that occur in co-infections. Therefore, the aim of our work was to develop a method for the detection of microsporidia based on a universal marker analysed by NGS.

Based on SSU rDNA sequences published in GenBank, we developed PCR primers that allow amplification of ca. 200-bp of the hypervariable V5 region from all microsporidian lineages. More than 200 adult mosquito females collected near Poznan, Poland, were individually screened for microsporidia by PCR with the use of double-indexed fusion primers for sequencing on the Ion Torrent PGM system. Sequence data from Sanger sequencing of the COI gene (DNA barcode) were used for mosquito species identification. Sequence data were analyzed using a custom workflow in Geneious R11 (Biomatters Ltd). All microsporidian species were confirmed by sequencing of the complete SSU rDNA; additionally, the PTP gene was used to determine *Encephalitozoon hellem* genotype.

Based on COI data, all mosquitoes were unambiguously assigned to eight species: *Ochlerotatus cantans* (77), *O. annulipes* (63), *O. sticticus* (13), *O. punctor* (11), *Aedes vexans* (20), *A. cinereus* (13), *Coquillettidia richiardii* (16) and *Culex pipiens* (3). Among all V5 region sequence reads (ca. 5,400,000), 15% were of the host origin, which

indicates the partial specificity of the developed primer set. Microsporidian DNA was found in more than half mosquitoes (54.17%). Based on the sequence data, the microsporidia were assigned to the 11 known species representing five genera: *Ambylospora*, *Enterocytospora*, *Nosema*, *Microsporidium* and *Encephalitozoon*. The predominant microsporidian species found in 50.46% individuals representing all mosquito species was the *Microsporidium* sp. 1199 (FN610845) known from molecular identifications in *Gammarus duebeni* (Crustacea, Amphipoda). Also we found this species as the most common in hosts that were co-infected with almost all detected species. Our method enabled us to detect, for the first time in mosquitoes, the *Encephalitozoon hellem* genotype 1A. This result implicates mosquitoes as potential sources of microsporidia infection for humans.

MICROBIAL CONTROL

Poster/Micr. Cont. Wednesday, 13.30. **MC-1**

Pathogens and parasites of bark beetles and leaf-rolling weevils in Bulgaria

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Beetles belonging to 12 species and collected from 15 localities (mainly coniferous stands) in Bulgaria were investigated for presence of pathogens and parasites. Two entomopathogens were detected for the first time – a microsporidium belonging to genus *Nosema* in *Pityogenes chalcographus* and the fungus *Beauveria bassiana* in *Attelabus nitens*. *Nosema* sp. was detected in the fat body of *P. chalcographus* with a prevalence of 0.9%. *B. bassiana* was found in 62% of the oak leaf-roller *A. nitens*. Morphological data and characteristics of *Nosema* sp. spores and conidia of *B. bassiana* are presented. Nematodes were detected in all 12 bark beetle species – in two of the bark beetle species, three species of nematodes (*Cryptaphelenchus diversispicularis, Parasitorhabditis subelongati* and *Parasitylenchus dispar)* were found. Individuals of nine nematode genera were found in the other ten beetle species: The found nematodes belong to the genera *Bovianema, Bursaphelenchus, Cryptaphelenchus, Neoparasitylenchus, Parasitylenchus, Parasitaphelenchus, Panagrolaimus, Parasitorhabditis* and *Sulphuretylenchus*. Nematodes were localized in the haemolymph and gut of the hosts, with prevalences ranging from 17.4% to 90%, depending on the host species. Four nematode genera were detected in *Orthotomicus erosus* and *Tomicus piniperda* and two genera each in *Dryocoetes autographus*, *I. acuminatus, I. sexdentatus, Orthotomicus laricis, Pityogenes chalcographus,* and *Pityogenes quadridens. Hylurgus ligniperda, I. typographus, Pityogenes conjunctus* and *Taphrorychus villifrons* were parasitized by one nematode genus. The results confirm the diversity of parasites among various species of bark beetles. Further studies on the effects of the found nematodes on the hosts are needed.

Poster/Micr. Cont. Wednesday, 13.30. **MC-2-STU**

Cajanus scarabaeoides inhibits *Helicoverpa armigera* larvae development

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The lepidopteran insect, *Helicoverpa armigera* (Hubner), is a polyphagous pest affecting major crops that results in economic losses of up to 300M USD/year. Host plant resistance is crucial for management of this pest in many crops. The cultivated pigeonpea, *Cajanus cajan* (L.) Millspaugh, lacks genetic variability for *H. armigera* resistance, which has led to an investigation to explore and harness the genetic variation of host plant resistance present in wild relatives of pigeonpea. We used *Cajanus scarabaeoides* (L.) Thouars, which has resistance mechanisms to pod borer, pod fly and possesses high protein content as well salinity and drought tolerance. A detached leaf assay was used to assess antibiosis and antixenosis resistance in *C. scarabaeoides*. An artificial diet supplemented with lyophilised leaf powder was employed to further investigate the antibiosis effect. Data on larval mortality and the weights of surviving larvae and pupae were collected on the 10th and 15th days after the start of feeding. Time taken for neonate larvae to pupate and for pupae to develop to moths were determined through daily observations. Our preliminary data showed that *H. armigera* larval weight was significantly reduced and larval development times were prolonged when reared on different accessions of *C. scarabaeoides* as compared to the susceptible check *C. cajan* (ICPL 87). Variable levels of resistance to *H. armig*era were observed on the different *C. scarabaeoides* accessions tested. To further investigate the

mechanics driving resistance mechanisms of *H. armigera* in *C. scarabaeoides*, proteomic and metabolomics analyses will be carried out. The *C. scarabaeoides* accession with the highest level of resistance will be used to increase the level of *H. armigera* resistance in commercial pigeonpea through interspecific hybridisation.

Poster/Micr. Cont. Wednesday, 13.30. **MC-3-STU**

Entomopathogenic fungi-mediated biological solution to control melon thrips *Thrips palmi*

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Melon thrips, *Thrips palmi* Karny (Thysanoptera: Thripidae) is one of the serious insect pests in many economic crops, and the management of this pest mainly relies on chemical insecticides. However, the use of chemicals for extended times caused insect resistance and environmental residual issues, and now the thrips management needs additional solutions. We need to consider alternative strategies which are less harmful to the environment and working on different target points. In this work, we isolated entomopathogenic fungi from soil, and identified with morphological and molecular biological methods, followed by a preliminary virulence assay against *Tenebrio molitor* larvae. Selected fungal isolates were adjusted to 1×10^7conidia/ml for an indoor virulence assay against *T. palmi* adults and highly virulent isolates were added to a thrip-pathogenic fungal library. Biological characteristics of an efficacious isolate were investigated by comparing with a previously commercialized fungal isolate *Beauveria bassiana* ERL836. This entomopathogenic fungal library could be used as a valuable resource for developing effective strains to control *T. palmi* in agricultural fields.

Poster/Micr. Cont. Wednesday, 13.30. **MC-4**

The role of *Beauveria pseudobassiana* in restoration of Mana Island and a rare native Flax weevil*, Anagotus fairburni*

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Restoration projects are typically designed to 'save' at-risk species. It is unusual for translocated species to become rampant and threaten other indigenous fauna and flora. But that is exactly the case when the flax weevil, *Anagotus fairburni,* was introduced to Mana Island in 2004 from Maud Island. The weevil eats both harakeke (*Phormium tenax*) and the smaller whaririki (*Phormium cookianum*) New Zealand flax bushes. The larvae live in the soil and feed on the flax roots, while the adults emerge at night and gnaw on the edges of the leaves. By 2013 large areas of flaxes had died and the weevil population was estimated to be in the tens of thousands as reported by Colin Miskelly in 2013, which in turn threatened the survival of the host plants and a rare endemic native gecko species reliant on the flaxes. On Maud Island, which is located on a similar latitude to Mana Island, the natural weevil population is limited and flax thrives. To determine if disease was contributing to weevil population stability on Maud Island, the weevils there were sampled in 2017, with a single diseased larva found. The causative agent was identified as (a strain of) *Beauveria pseudobassiana*. The potential of introducing the fungus to Mana Island as a natural biocontrol agent for the translocated weevil populations was considered. However a subsequent survey of flax weevil larvae and adults from Mana Island showed *B. pseudobassiana* was already present at the translocation site. Bioassays of the fungus against larvae and adults of the weevil confirmed direct infection. The natural infection pre-empted the need to make a decision to introduce a naturally occurring bio-control but does raise questions for future restoration projects, including why the fungus is not acting as a control at the weevil release site.

Poster/Micr. Cont. Wednesday, 13.30. **MC-5**

Synergistic insecticidal activity of dsRNA specific to insulin signalling components with *Bacillus thuringiensis*

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Two insulin-like peptides (ILPs) have been identified in *Maruca vitrata* (Lepidoptera: Crambidae) and are expressed in both larval and adult stages. Furthermore, insulin/IGF signalling (IIS) mediates nutrient signalling after feeding and promotes larval growth and adult reproduction. Injection of a porcine insulin stimulated larval development in a dose-dependent manner. In contrast, RNA interference of IIS component genes such as insulin receptor (InR), Forkhead box O (FOXO), Akt, and target of rapamycin (TOR) impaired larval development and resulted in significant mortality. Four dsRNAs specific to the four IIS components were expressed in HT115 bacterial expression system using L4440 expression vector. The transformed bacteria expressed quite large amount of dsRNA. Feeding the transformed bacteria also resulted in significant mortality. To enhance the

insecticidal activities of the dsRNA-expressing bacteria, a bacterial mixture with *Bacillus thuringiensis* (Bt) was devised. Among four Bt strains, *B. thuringiensis kurstaki* (BtK) was effective to kill *M. vitrata*. Mixture treatment of dsRNA-expressing *E. coli* and BtK exhibited a synergistic insecticidal mortality.

Poster/Micr. Cont. Wednesday, 13.30. **MC-6-STU**

Two new *Bacillus thuringiensis* strains toxic against *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

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The attack of insects in monocultures have generated great concern worldwide, especially armyworms of the genus *Spodoptera*. As an alternative to chemical pesticides, insecticidal toxins from the bacterium *Bacillus thuringiensis* have been used in formulated products and transgenic plants for the control of these insects. However, the emergence of insects resistant to these toxins has demonstrated the importance of studies for the identification of new toxins and toxin-receptor interactions in the gut of target insect pests, thus assisting in insect resistance management. Therefore, this study aimed to identify the insecticidal (Cry) proteins of two *B. thuringiensis* strains, toxic to *S. frugiperda* (Lepidoptera: Noctuidae), considered a major agricultural pest. Two strains, named Bt-MTox 2638-1 and Bt-MTox 2974-11 were selected for showing 100% mortality of *S. frugiperda* 7 days after inoculation. Their DNA was extracted and amplified through PCR using universal primers for *cry* genes. The amplicons were extracted from agarose gels after electrophoresis, purified and cloned. Successful clone insertions were then sequenced. From the sequencing of the two *Bt* isolates, two genes, one similar to *cry1J* for Bt-MTox 2638-1 and one similar to *cry1D* for Bt-MTox 2974-11 were identified. The results of this study show two new *Bt* proteins with potential for use in biological control, especially for their possibility to possess modes of action different from the currently used Cry toxins. In order to ensure their applicability, additional investigations of those two toxins are necessary, focusing on the pure proteins obtained after insertion of the identified *cry* genes into *E. coli*. Analyses such as protein profiling and conformation, binding assays with receptor sites in the insect midgut and cross-resistance assays with insects resistant to other Cry toxins are crucial for further development of those strains as biological control agents. Additionally, it is important the undertaking of resistance selections for the identification of possible resistance genes and their possible cross-resistance to toxins already used in the market.

Poster/Micr. Cont. Wednesday, 13.30. **MC-7**

Characterization of chitinases of *Beauveria bassiana* (Bv062) induced in semisolid-state fermentation

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Beauveria bassiana chitinases are an important cuticle-degrading group of enzymes implicated in the pathogenesis caused by this fungus in insects, which makes them potential targets for biotechnological developments in pest control. The aim of this study was to characterize chitinases induced during *B. bassiana* conidia production. *B. bassiana* isolated from *Diatraea* sp. larvae in Colombia, was inoculated on a semisolid medium based on oat (C/N of 19:1) and compared with the same medium supplemented with wheat bran (0.1%). In both cases, conidia were harvested after 14 days of incubation at 25°C and then were characterized by determining chitinolytic activity on 4-Nitrophenyl N,N-diacetyl-b-D-chitobioside, 4-Nitrophenyl N-acetyl-b-D-glucosaminide and 4-Nitrophenyl b-D-N,N,N-triacetylchitotriose substrates. Conidia showed exo-chitobiosidase and exo-B-N-acetylglucosaminidase activity, while the endochitinase activity was very low. The exochitinase activities were increased by the supplemented media. To evaluate the expression of enzymes, three fragments with chitinases motifs were amplified by using RNA extracted from conidia and designed ad-hoc primers. Bioinformatics analyses revealed 1044, 2867 and 1453 bp fragments. The corresponding proteins presented the typical catalytic domain of chitinases belonging to family 18, one of them with a chitin binding domain, and the others presented secretory domains. These preliminary results show a way of new strategies to enhance virulence factors of entomopathogenic fungi.

Poster/Micr. Cont. Wednesday, 13.30. **MC-8**

In-plant protection from the insect pest *Helicoverpa armigera* by trans-kingdom RNAi

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Helicoverpa armigera, the cotton bollworm, is a major insect pest for a wide range of agricultural crops. It causes huge yield losses not only through feeding damage but also by increasing the crop's vulnerability to bacterial and fungal infection. *H. armigera* has evolved substantial resistance to most of the available classes of chemical insecticides, prompting the development of transgenic crop plants with alternative insect-resistance-conferring mechanisms. For example, transgenic crops producing *Bacillus thuringiensis* (Bt) toxins have been very successful. However, there is still a concern about insect populations emerging with resistance to such biopesticides. Novel strategies that give protection as effective as conventional insecticides, without affecting the environment, need to be continuously developed and improved. Trans-kingdom RNA interference (TK-RNAi), when double-stranded or hairpin RNA are expressed in transgenic plants to silence essential genes within herbivorous pests, has emerged as a promising strategy for managing crop pests. We describe new ways of delivering and improving plant protection against *H. armigera* through TK-RNAi.

Poster/Micr. Cont. Wednesday, 13.30. **MC-9-STU**

Chitinase of *Trichoderma koningiopsis* to enhance the insecticidal activity of *Beauveria bassiana* to control the sugar cane borer *Diatraea saccharalis*

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Chitinases are key enzymes involved in the virulence of entomopathogenic fungi and its expression can be induced by manipulating the culture media composition and fermentations conditions. It is thought that these enzymes are also produced by antagonistic fungi such as *Trichoderma* spp. against fungal plant pathogens. The aim of this study was to evaluate the effect of combining extracellular N-acetylglucosaminidase produced in solid-state fermentation from an antagonistic strain of *Trichoderma koningiopsis,* with conidia of a *Beauveria bassiana* strain previously selected for its pathogenicity against different species of *Diatraea* spp. An isolate of *T. koningiopsis* Th003 was grown on rice-wheat bran and a crude extract was obtained from the substrate submerged in tween 80 at 1% (p/v). A N-acetylglucosaminidase enzyme (EC 3.2.1.52) was partially purified in two steps using a Q-sepharose chromatography followed by a Sephacryl S-200 column chromatography. The optimum activity was observed at pH 5 and 55 °C using p-nitrophenyl-β-D-glucosaminide (pNGlcNAc) as substrate. Michaelis constant (K_m) and maximal velocity (V_{max}) values for pNGlcNAc hydrolysis were 0.28 mg/mL and 0.8 µmol/min, respectively. The topical application on larvae of purified chitinase caused 1.6% larval mortality at 6 days after application. In the other hand, the enzyme applied simultaneously with *B. bassiana* conidia caused 60% mortality in comparison with 40% obtained with the control using pure conidia. The enzyme application also reduced conidia median lethal time (LT_{50}) from 6.8 days to 5.1 days, suggesting a synergistic effect possibly related with a faster degradation of insect cuticle, improving the penetration process.

Poster/Micr. Cont. Wednesday, 13.30. **MC-10-STU**

Evaluation of additives to induce enzymatic activity of *Beauveria bassiana* conidia and improve insecticidal activity against *Diatraea saccharalis*

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One important step during the infection process of entomopathogenic fungi is the degradation of the insect cuticle by the secretion of extracellular enzymes such as lipases, proteases and chitinases. Therefore, these enzymes are considered important virulence factors and their expression, excretion and activity has been the objective of diverse studies looking to improve the performance of different biocontrol agents. In order to improve the virulence of *Beauveria bassiana* Bv062 against *Diatraea saccharalis*, conidia were produced using semi-solid state fermentation with a previously selected medium based on oat. The medium was supplemented with: soy protein, wheat bran and chitosan at different concentrations (0.1%, 0.5% and 1.0%). Conidia were harvested after 14 days of incubation at 25 °C and characterized by determining germination, enzyme activity and biocontrol activity. In general, lipases, total proteases, protease Pr1 and N-

acetylglucosaminidase were induced by all the additives at all evaluated concentrations. The lethal times fifty (LT_{50}) and ninety (LT_{90}) were reduced by approximately 2 days by supplementing the oat based medium with soy protein (1%) and wheat bran (0.1% and 0.5%), in comparison with the LT_{50} and LT_{90} of conidia produced on non-supplemented oat with 8.0 and 16.8 days, respectively. Proteases demonstrated to be very important virulence factors for *B. bassiana* Bv062 against *D. saccharalis* and its expression and accumulation in produced conidia was induced when the culture medium was supplemented with a high protein additive.

Poster/Micr. Cont. Wednesday, 13.30. **MC-11-STU**

Selection of an adequate substrate to produce high quality conidia with a Colombian isolate of *Beauveria bassiana* to control the sugar cane borer *Diatraea saccharalis*

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The isolate of *Beauveria bassiana* Bv062 was previously selected due to its activity against larvae of three species of *Diatraea*, with efficacies of up to 60% under laboratory conditions. In order to continue developing this isolate as a potential biopesticide, two substrates for semisolid-state fermentation were evaluated with the aim to produce high quality conidia. *B. bassiana* was inoculated on two semisolid media based on oat (C/N of 19:1) and rice (C/N of 43:1), which were adjusted to different levels of water activity (a_w): 0.95, 0.97 and 0.99. The fungus was not able to grow when a_w was 0.95 and 0.97, but rapidly colonized and sporulated the culture medium surface when a_w was 0.99. Conidia yield and accumulation of polyols were high with the medium based on oat and rice. Conidia produced with the medium based on oat also presented higher germination before and after 30 days of storage at 30 °C (94% and 81%, respectively) than conidia on rice (94% and 74%, respectively). Faster mortality (LT_{90}) on *D. saccharalis* larvae was also obtained when Bv062 was grown on oat (14.9 days) in comparison with rice (18.5 days), which could be related with higher protease and chitinase activity. The obtained results allowed the selection of the oat-based medium for the production of Bv062 conidia with better eco-physiological performance.

Poster/Micr. Cont. Wednesday, 13.30. **MC-12**

Evaluation and monitoring of maize crops in Brazil expressing the Cry1F toxin from *Bacillus thuringiensis* in the control of *Helicoverpa armigera* and *Spodoptera frugiperda*

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Proteins derived from the entomopathogenic bacterium *Bacillus thuringiensis* (Bt) are able to bind to receptors in the midgut of susceptible insects, such as aminopeptidase-N (APN) and alkaline phosphatase (ALP). Maize transgenic plants expressing the toxin Cry1Ab, Cry1Ac and Cry1F from Bt are highly attacked by important agricultural pests such as *Spodoptera frugiperda* and *Helicoverpa armigera*. The monitoring of these species in the field allows the decision making in a timely manner, avoiding the selection and the increase of resistant populations of caterpillars. The presence and activity of ALP and APN from susceptible *S. frugiperda* (SfLab), resistant *S. frugiperda* (Sfr), susceptible *H. armigera* (HaLab) and *H. armigera* collected in the field (HaC) were analyzed. The presence of brush border membrane vesicles (BBMV) in *S. frugiperda* larvae was analyzed by determining the specific activity of ALP and APN and also by Western blot with *S. frugiperda* ALP antibodies. The ALP receptors of the Sfr population presented low levels of expression in all analyzes when compared with SfLab. Differences in detection levels were not observed for HaLab and HaC, suggesting that resistance of *S. frugiperda* insects may be involved with ALP receptors and that *H. armigera* collected in the field are still unchanged in these types of receptors. APN receptors showed no differences in expression levels in any of the four populations used. The modified proteins Cry1FN507A and Cry1AbN514 had increased toxicity in *H. armigera* populations. Bioassays with modified proteins against *S. frugiperda* demonstrated insecticidal activity similar to wild-type Cry1Ab. These data represent an alternative control of *S. frugiperda* and *H. armigera* in the use of these modified proteins in the field.

Poster/Micr. Cont. Wednes., 13.30. **MC-13-STU**

Identification and characterization of novel juvenile hormone antagonists from *Streptomycetes*

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Actinomycetes have been known to produce variety of bioactive metabolites that include antibiotics and plant growth factor. Also, insecticidal compounds such as avermectin and tetranectin have been reported from actinomycetes. Recently, we have developed high-throughput juvenile hormone antagonist (JHAN) screening system using yeast-two hybrid system. In this study, to identify novel IGR-based insecticidal candidates, 1,274 actinomycetes culture filtrates were tested for their JHAN activity. Among 34 culture filtrates showing JHAN activity, IMBL-263 showed high level of insecticidal activity against 3rd instar larvae of *Plutella xylostella*. This isolate was identified as *Streptomyces anulatus* using 16S rRNA gene sequencing. When the culture filtrate of IMBL-263 was extract with hexane and ethyl acetate depending on the polarity of the solvent, ethyl acetate extract showed both JHAN and insecticidal activity. These results suggested that secondary metabolites from the *S. anulatus* might be useful for development of novel environmentally benign insecticides.

Poster/Micr. Cont. Wednesday, 13.30. **MC-14**

Microbial control of the western grapeleaf skeletonizer

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The western grapeleaf skeletonizer (WGLS), *Harrisina metallica* (Lepidoptera: Zygaenidae) is a destructive pest of grapes in California. Organic vineyards are especially at risk and uncontrolled populations can destroy vineyards resulting in significant losses. Early instar WGLS larvae feed on the lower leaf surface and late instars feed on the entire leaf lamina leaving major veins. Assays were conducted to evaluate the efficacy of Entrust (spinosad), two California isolates of *Beauveria bassiana* (SfBb1 or ARSEF 8318) and *Metarhizium anisopliae* (GmMa1 or ARSEF 8319), Agree (*Bacillus thuringiensis* subsp. *aizawai*), Deliver WG (*B. thuringiensis* subsp. *kurstaki*), and Neemix 4.5 (azadirachtin). All the commercial pesticides were used at the recommended field application rates in 100 gallons of water. Entomopathogenic fungi were used at 1.0E+8 conidial/ml rate. Dyne-Amic surfactant was used at 0.125% vol/vol in all treatments. Each treatment had five 4-5 instar larvae and exposed to 1 ml of respective treatment solution. Untreated controls were sprayed with the surfactant solution. Treatments were replicated four times and assays were repeated twice. Total mortality in larvae was 37.5, 70, 81.3, 85, 92.5, 100, and 100% in control, Deliver, Agree, Neemix, *B. bassiana*, *M. anisopliae*, and Entrust treatments, respectively. All treatments worked fairly well, but based on the corrected mortality (using Abbott's formula), Entrust (100%) and *M. anisopliae* (100%) were highly effective followed by *B. bassiana* (81.3%), Neemix (78.3%), Agree (73.3%), and Deliver (45%). Our study demonstrates the potential of non-chemical control options for managing WGLS. Both California entomopathogenic fungal isolates have good pest control potential.

Poster/Micr. Cont. Wednesday, 13.30. **MC-15**

Selection and Characteristics of Entomopathogenic Fungi for Microbial Control of *Plutella xylostella*

Ji Hee Han, Jae Yoon Kim, Moran Lee, Hye Ju Jeong, Dayeon Kim, Seongho Ahn and Sang Yeob Lee

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Diamondback moth, *Plutella xylostella* is the most damageable pest in cruciferae plants including chinese cabbage. The main reason of that is the moth has short life cycle as 20~25 days, and occur 9~12 generation annually and its population increases faster compare to other lepidopteran pests. Moreover its control is very difficult due to the development of its resistance to insecticides. In this study, *Metarhizium anisopliae* FT319, *Isaria fumosorosea* FT337 isolates were used among 10 isolates collected from soil by insect-bait method and followed by bioassay. Spraying of *M. anisopliae* FT319 and *I. fumosorosea* FT337 (10^7 conidia/ml) against third instar larva of *P. xylostella* generated 5days after treatment average 90% and 75% of mortality respectively at laboratory. To determine the suitable treatment temperature to control the pest using the two isolates, we investigated the activities of the two fungi between 15℃ and 35℃. *M. anisopliae* FT319 and *I. fumosorosea* FT337 showed highest mortality at the 25℃ which were 87% and 100% respectively, also good mycelium growth of the two isolate happened at 25℃ for *M. anisopliae* FT319 and *I. fumosorosea* FT337 respectively.

Poster/Micr. Cont. Wednesday, 13.30. **MC-16**

Entomopathogenic Fungi for Dual Control of Thrips and Plant Fungal Disease

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A lot of plant diseases and pests infest crops during crop production and reduce yield and quality of crop. Various control agents were applied to control plant disease and pest. But pesticide resistance which is caused by repeated applications makes it more difficult to control pest and disease. In this study, as part of effort to reduce use of pesticide we selected microbial for dual control both pest and plant pathogen. We selected fourteen entomopathogenic fungi have anti-fungal activity against plant pathogens, *Collectotrichum acutatum* and *Phytophthora capsici* and investigated their insecticidal effect against *Thrips palmi.* Among the fourteen isolates, 3 isolates showed high virulence against *Thrips palmi* as 60.0~90% mortality 5 days after treatment.

Poster/Micr. Cont. Wednes., 13.30. **MC-17-STU**

Laboratory and field evaluation of entomopathogenic fungi and bacteria for the control of *Rachiplusia nu* (Guenée) (Lepidoptera: Noctuidae) in soybean (*Glycine max* (L.) Merrill).

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Rachiplusia nu is a lepidopteran specie that causes serious economic losses in the most important cultivated crop in Argentina, soybean. Biological control using entomopathogenic fungi and bacteria is a promising alternative to chemical insecticides being used for their control. The pathogenicity formulations of *Beauveria bassiana* strain (LPSc 1098) and *Bacillus thuringiensis* strain (Ale 10) were tested in the laboratory against larvae of *R. nu*. Larvae L3 were sprayed with conidial suspensions and spores. Conidial suspensions and spores were pathogenic to *R. nu* larvae, causing mortality of 96.6% and 73.3%, respectively, in lab conditions.
In the field, four treatments including control, chemical insecticide, spores and conidial suspensions (F3) and conidial suspensions (F4) were applied on soybean. The treatments significantly reduced the number of larvae in all experimental units compared to the control, causing mortality of 97,1%, 82,4% and 61,8% respectively. The use of two microorganisms is highlighted by providing two mechanisms of action by contact in the case of the fungus and by ingestion in the case of bacteria. These mechanisms of combined action contribute to the non-development of resistance of the insect. Results of the present study indicate that biological control can be used for the management of *Rachiplusia nu*.

NEMATODES

Poster/Nematodes. Wednesday, 13.30. **NE-1-STU**

Diversity of entomopathogenic nematodes and their symbiotic bacteria in Australian soils and their interaction with Queensland fruit fly (*Bactrocera tryoni*)

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Queensland fruit fly, *Bactrocera tryoni* (Tephritidae), is Australia's worst horticultural pest damaging fruit and limiting domestic and international market access. It originates from tropical and subtropical Australia and has invaded temperate environments. It develops in maturing fruit of many plant species and pupates in the soil. Several chemical insecticides for fruit fly control have recently been abolished, and there are few alternative control strategies. Natural enemies of insect pests may provide a solution. Entomopathogenic nematodes (EPNs) from the families of Steinernematidae and Heterorhabditidae are important biocontrol agents of insect pests with life stages in the soil; however, their diversity and interactions with symbiotic bacteria and tephritid fruit flies in Australia has not been investigated. Australia is a continent with diverse climates and soils which could host yet unknown EPNs and parasitic nematodes which might be more virulent and effective against *B. tryoni* than commercially available EPNs. Collecting locally occurring EPNs and other insect parasitic nematodes may uncover isolates with a higher efficacy due to their better adaptation to local climate and population regulators. In a preliminary experiment, we sampled soil from orchards near Richmond, New South Wales, and baited for nematodes with larvae of mealworm (*Tenebrio molitor*), *B. tryoni* and wax moth (*Galleria mellonella*). EPNs were found in only 9 of 60 and 2 of 60 for *T. molitor* and *B. tryoni*, respectively. No EPNs were detected when baited with *G. mellonella*. Based on preliminary morphological characterization, *T. molitor* captured *Heterohabditis* spp., whereas *B. tryoni* captured a diversity of nematodes. The latter were all found dead inside *B.*

tryoni cadavers and were unable to emerge. Furthermore, mealworm emerged EPNs did not survive temperatures below 6°C. Molecular and morphological characterization of nematode species and bacterial symbionts is ongoing. The EPNs will then be evaluated for their efficacy against larval and pupal stages of *B. tryoni*. Analysis of EPN and bacterial strain diversity collected from different regions of Australia (temperate, subtropical and tropical regions) and different management regimes will be performed next to contrast environmental drivers of EPN communities, and to obtain EPN with higher virulence against *B. tryoni* across its entire distribution in Australia.

Poster/Nematodes. Wednesday, 13.30. **NE-2**

Isolation of cell wall encapsulated or purified Cry5B crystals from asporogenous *Bacilli* for use as a anthelminthic drug

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Soil transmitted helminthiases are some of the most common neglected tropical diseases that affect 1.5 billion people globally, predominantly children and women of child bearing age in poor and developing countries. WHO recommends periodic deworming using small molecule anti-helminthic drugs in endemic areas to counter the health effects of increased worm burdens; abdominal pain, diarrhea, physical and developmental stunting and nutritional deficiency. Such mass administration often leads to the rise of parasite resistance or tolerance and thus a need for a new cure with minimal side effects is imminent. In this context, invertebrate-specific bacterial pore-forming proteins are excellent candidates that have evolved naturally to disrupt host membranes, been widely used as insecticidal pesticides (e.g., Bti, transgenic corn) and also are part of our diet. Crystal protein, Cry5B, from *Bacillus thuringiensis* (Bt) was earlier shown to be efficacious against *Ancylostoma ceylanicum* (hookworm) and *Ascaris suum* (roundworm). However, in these experiments spore-crystal lysates were used to test toxicity to worms. To avoid the dissemination of live spores and the potential enterotoxicity with Bt, we genetically engineered *Bt* to form a non-sporulating Bacillus with Cytosolic Crystal (BaCC). After an inactivation step to kill live vegetative bacteria, the Inactivated BaCC (IBaCC) was tested for bioactivity against *Caenorhabditis elegans* and hookworms *in vitro*. Interestingly, the IBaCC bacterial cell ghosts containing Cry5B crystals were highly toxic to the worms, while mutant inactivated *Bacillus* lacking Cry5B had no effect on worms. These results support the continued development of IBaCC as a new cost-effective candidate for veterinary and livestock deworming applications. Formulation development work is ongoing to optimize oral delivery. To isolate Purified Cry5B Crystals (PCC) for pharmaceutical applications, we devised a two-step, simple and scalable process that generated > 90% pure, bioactive Cry5B protein crystals. Taken together, we have developed two forms of Cry5B – encapsulated inactive bacteria (IBaCC) and purified Cry5B crystals with potent anti-helminthic properties. These two new crystal protein production methods can also be extended to other crystal proteins produced in asporogenous hosts.

Poster/Nematodes. Wednesday, 13.30. **NE-3**

Characterization of the heat shock protein 90 gene of *Heterorhabditis bacteriophora* and its expression in response to different temperature stress.

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Heat shock proteins belong to a protein family that are rapidly synthesized in response to a series of environmental stressors. Among all HSPs, HSP90 is the most conserved and abundant in cells and is involved in response to temperature stress. Temperature affects life-stage development and behaviour of entomopathogenic nematodes, with different species and strains having optimum temperatures for penetration, feeding, survival and reproduction. Thus, temperature is an important factor in controlling generation time of EPN. In the present study, the full-length cDNA and the corresponding gene of HSP90 of *Heterorhabditis bacteriophora* were cloned and sequenced and its expression in response to three different temperatures was also investigated. The full-length *Hb-hsp*90 cDNA contained an ORF of 2139 bp encoding a polypeptide of 713 amino acids. The deduced amino acid sequence of Hb-Hsp90 showed high similarity with other known HSP90s. The gene consists of 12 exons and 11 introns, an expanded gene structure compared to other nematode *hsp*90 genes. *Hb-hsp*90 gene was constitutively expressed in all developmental stages of *H. bacteriophora* but at higher level in adult males and females. Larvae of *Galleria mellonella* were infected with infective juveniles (IJs) of *H. bacteriophora* and exposed at 12°C, 23°C and 30°C for 10 days in order to explore the impact of adverse temperature on reproduction and infectivity of *H. bacteriophor*a and on *Hb-hsp*90

expression in IJs and adult stages. Results obtained in this study are reported and discussed.

Poster/Nematodes. Wednesday, 13.30. **NE-4**

Trait derioration rate of entomopathogenic nematodes and selection of superior inbred lines

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A number of entomopathogenic nematode (EPN) species belonging to the families Steinernematidae and Heterorhabditidae have proven to be safe and effective biocontrol agents for suppression of insect pests. An important step in the development of bio-insecticidal nematodes is *ex situ* preservation and mass production. However, their exploitation is limited by beneficial trait deterioration triggered by artificial manipulation *ex situ.* The use of homozygous inbred lines have proved to stabilize EPNs. We hypothize that rate of trait deterioration will vary amongst different species and strains and there will be substantial variation of inbred lines for different beneficial traits. Trait deterioration rate of eight strains representing five species (*Heterorhabditis bacteriophora* HbHp88, *H. bacteriophora* VS, *H. indica* Hom1, *Steinernema carpocapsae* All, *S. carpocapsae* Cxrd, *S. feltiae* SN, *S. glaseri VS* and *S. glaseri* Sg11a+7b) was measured by assessing them vs. their wild type for heat tolerance, virulence and reproduction capacity. Homozygous inbred lines of the two strains of *H. bacteriophora* (HbHp88 and HbVS) were generated and evaluated for the same traits (heat tolerance, virulence and reproduction). Variation in trait deterioration rate of the different strains and variation in performance of the inbred lines was observed. Homozygous inbred lines carry potential for stabilization of EPNs for future exploitation as biopesticides.

Poster/Nematodes. Wednesday, 13.30. **NE-5-STU**

Genetic diversity of the biocontrol nematode *Deladenus siricidicola* in Australia and New Zealand

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Deladenus siricidicola is a parasitic nematode of the woodwasp *Sirex noctilio*, which is invasive in the Southern Hemisphere where it infests and kills pine trees. In New Zealand, *D. siricidicola* was accidentally introduced along with *S. noctilio*. In Australia, however, as in other Southern Hemisphere countries, *D. siricidicola* has been introduced deliberately as a biological control agent. These releases are based largely on a single strain, which influences the genetic diversity in populations of the nematode in those regions. Low levels of genetic diversity could impact the effectiveness of the nematode to adapt to different environmental conditions and different host populations. A previous study on the genetic diversity of *D. siricidicola* in some regions of the Southern Hemisphere revealed extreme homozygosity in populations linked to biological control programs. No information is, however, available from field collected populations of the nematode in Australia and/or New Zealand where different strains were released or accidentally introduced. This study, therefore, aimed to characterize the level of population genetic diversity and structure of *D. siricidicola* in these two countries using mitochondrial COI sequence data and 11 previously developed microsatellite markers. These data were compared to that of a recent study that applied these markers to populations of the nematode from countries in South and North America, South Africa and Europe. Three previously defined lineages (lineage A, B, and C) were also identified in this study from Australia, and two lineages (lineage A and B) from New Zealand. Interestingly, an admixed population (between lineage A and B) was also found in Australia. Finally, a new lineage (D) was revealed in Australia through both COI sequence analysis and microsatellite markers. The level of diversity in populations of *D. siricidicola* in Australia appears to be higher than that from other regions in the world. This knowledge can be used to further select strains for efficiency or for use in different environments where different populations of *S. noctilio* are a problem.

Poster/Nematodes. Wednesday, 13.30. **NE-6-STU**

Improvement of oxidative stress tolerance and longevity of the entomopathogenic nematode *Heterorhabditis bacteriophora* through genetic selection

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Heterorhabditis bacteriophora Poinar (Rhabditomorpha: Strongyloidea) is among the few entomopathogenic nematodes (EPN) species currently produced at industrial scale used for insect pest management. For industrial production, the dauer juveniles (DJs) are propagated in bioreactors in large volumes (up to 40 m^3) in e-nema GmbH (Schwentinental, Germany). Subsequently, DJs are stored, formulated, transported and applied in the field. However, from production to application, various environmental stress factors (heat, desiccation, UV-radiation, hypoxia and oxidative stress) reduce DJ longevity and virulence of EPN species. Understanding the genetic component of the stress responses in *H. bacteriophora* and improving longevity are important research tasks. In this study, we screened 40 wild type strains and lines collected from different geographical locations for their mean time survival for with and without oxidative stress inductions at 25°C and 7°C. We determined a high variability among strains of this species and a high heritability for oxidative stress tolerance (h^2> 0.9). We also found that oxidative stress tolerance is correlated with DJ longevity and therefore can be used as a predictor for DJ longevity, permitting a selection process within a shorter testing period. To further improve tolerance to oxidative stress and longevity, several genetic crosses, EMS-mutants and homozygous inbred lines were produced. These genetic crosses, EMS-mutants and homozygous inbred lines were found to have a higher tolerance to oxidative stress, improved longevity in water and higher infectivity against mealworm (*Tenebrio molitor)* compared to their respective parental (AU1 and HU2), donor (IL3) and commercial (EN01) lines. Furthermore, the oxidative stress-responsive transcriptome of two contrasting lines was performed using Massive Amplification of cDNA Ends (MACE) and candidate genes were screened for polymorphisms. Our *de novo* transcriptome assembly generated a total of 20,022 transcripts during the early stages of oxidative stress. More than 500 SNPs have been detected between stress-tolerant (HU2-IL1) and stress-sensitive (PT1-IL1) inbred line. PCR-based KASP markers were derived from relevant transcripts and were tested in *H. bacteriophora* materials. Significant correlation between genotype and phenotype was determined for a subset of KASP markers. This research will facilitate a marker-assisted selection and further breeding activities to prolong shelf-life of the nematodes.

Poster/Nematodes. Wednesday, 13.30. **NE-7**

A survey of the parasitic nematodes of invertebrates in Mindanao Island, the Philippines

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Parasitic nematodes of dominant species of invertebrates were studied in Mindanao island, the Philippines. A total of six provinces with different levels of disturbance were selected as the sampling areas – Davao del Norte (Samal island), Davao City (Gemphsimany garden, Marilog District), Bukidnon (CEDAR, Impasug-ong), Misamis Oriental (Initao), Camaguin (Mambajao seashore) and Lanao del Norte (Tinago falls, Linamon). Invertebrate animals such as earthworms, terrestrial mollusks, diplopods and some groups of insects (i.e.cockroaches, mole-crickets, Passalidae and Scarabaeidae beetles) were collected and dissected. The most widespread species of parasitic nematodes were collected and fixed in 4-6% formaldehyde for morphological studies and in 70% ethanol for DNA extraction and amplification of characteristic nucleotide sequences. Up to now the nucleotide sequences were obtained for the nematodes of the genus *Heth* (Ransonematoidea: Hethidae). The level of nucleotide differences between the *Heth* nematodes from three localities in Mindanao and *Heth* nematodes from Indonesia and Viet Nam was evaluated. Very few parasitic and necromenic nematodes were found in terrestrial mollusks. The parasitic nematodes of earthworms were mainly found in urban habitats, whereas majority of earthworms collected in natural habitats were free of nematode infection. Additionally, the earthworms collected near Tinago Falls, Linamon were found to be invaded by the nematodes of genus *Synoecnema* (Drilonematoidea: Ungellidae). The fauna of Passalidae beetles is very rich in Mindanao, however, only some representatives of the genus *Hystrignathus* (Oxyurida: Hystrignathidae) in beetles collected in Gephsimany Garden were identified.

Poster/Nematodes. Wednesday, 13.30. **NE-8**

Entomopathogenic nematode, *Steinernema kraussei* – the first recorded from Korea and temperature effect on Ulleungdo strain

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Entomopathogenic nematode, *Steinernema kraussei* which was firstly isolated from Gwangju, Yeongju, Jeongeup, and Ulleungdo island in Korea. Phylogenetic relationship of isolated species using ITS and effect of temperature on pathogenicity, penetration activity, and progeny production of *S. kraussei* Ulleungdo strain were investigated. In the temperature effect on *S. kraussei* Ulleungdo strain, pathogenicity was faster at high temperature although 100% mortality was shown even at 15℃ in 4 days. Numbers of penetrated infective juveniles into the host was highest at 15℃. Sex ratio of *S. kraussei* according to temperature was 6:4 except at 20℃. Numbers of progenies were also highest at 15℃ as 51,221, but not statistically significantly different from 20℃ and 25℃. *S. kraussei* is cold-active entomopathogenic nematode. Thus, *S. kraussei* can be highly available against insect pests occurring in cold places and seasons in Korea.

Poster/Nematodes. Wednesday, 13.30. **NE-9**

Slug parasitic nematode presence in Delaware

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Over the years, slugs have been a recurrent problem in the Mid-Atlantic region and Delaware. Synthetic molluscucidals offer a certain level of control but abiotic conditions dramatically limit their efficacy in the field, often leaving growers with very limited option to control this problematic invertebrate pests. Years with heavy slug pressure, fields must be replanted to achieve a decent seasonal yield. In Europe, growers can use a registered slug parasitic nematode (*Phasmarhabditis hermaphrodita*) to effectively control slugs in their problem fields and maintain yield. Despite one isolation of the species in California, this product is unfortunately not available yet in the USA where growers still yearly face major slug infestations. We therefore undertook an slug survey in the state of Delaware to isolate slug parasitic nematodes and hopefully isolate *P. hermaphrodita*. From hundreds of slugs captured from soybean fields, we have currently isolated 47 populations of slug parasitic nematodes. Proper identification has yet to be done, but some of the isolates displayed similarities with *P. hermaphrodita.* This could ease the registration of this nematode as a biocontrol agent in the USA and eventually provide growers with a highly potent tool to efficiently control slugs.

Poster/Nematodes. Wednesday, 13.30. **NE-10**

Cloning, expression and insecticides activity of ATP binding protein in *Aedes aegypti* against Bt

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Bacillus thuringiensis subsp. *israelensis* (Bti) is one of the widely used mosquitocidal microbiopesticides for its high toxicity to many mosquitos like *Culex, Aedes* etc. The ATP binding protein is prevalently appeared in insects which has been identified to have some connection with the process of Bti toxin reaction with insects. So far, we still have no idea with the function about the ATP binding protein in mosquito. In order to clarify the function of it in the process of Bti toxin reaction in *Ae. aegypti*, the gene of the ATP binding protein was cloned and expressed. Far-western blot and ELISA was taken to identify the interaction of ATP binding protein and the toxin Cry11Aa. A bioassay was taken in the presence and absence of the ATP binding protein which showed that with the increase of the ATP binding protein, the mortality would enhance. These suggest that the ATP binding protein can modulate the toxicity of Cry11Aa toxin to mosquito by binding to the toxin.

VIRUSES

Poster/Viruses. Wednesday, 13.30. **VR-1**

Baculoviruses as a tool for generating stable and effective sub-unit vaccines

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Baculoviruses have been widely used for recombinant protein expression since the early 1980's. Generating high-yield recombinant proteins from a vector that is incapable of replication in mammalian cells is a very attractive tool for developing safe and effective vaccines. The focus of

this project was to develop a sub-unit vaccine against African horse sickness virus (AHSV), which is an infectious, non-contagious insect vector-borne disease of equids. There are nine AHSV strains and the prevention of the disease is dependent on an efficient vaccination regime. Currently there are only live-attenuated vaccines available, however; they can only be used in endemic regions due to safety concerns. As AHSV replicates in insect cells during its transmission to a mammalian host, the baculovirus insect cell expression system becomes very advantageous for the production of correctly-folded AHSV proteins. VP2, the outercapsid protein of AHSV, has long been established to be the main target for generating virus neutralizing antibodies, which is crucial for protection. In this project, a recombinant baculovirus was used to express histidine-tagged VP2 in TnHi5 cells under the control of polyhedrin promoter. Mice were immunized to test the immunogenicity of VP2 both from crude cell lysates and as a purified protein. Indirect ELISA and monolayer protection assays showed that VP2 from both preparations were very immunogenic. Furthermore, a single dose of purified VP2 was shown to provide a complete clinical protection in an AHSV mouse model. This is an added value in the case of a multi-strain disease target as it provides promise for the generation of efficient multivalent vaccines. In addition, purified VP2 was shown to retain sufficient immunogenicity after long term (12+ months) storage at 4°C post-production, which is very advantageous for the generation of a cost-effective vaccine.

Poster/Viruses. Wednesday, 13.30. **VR-2**

Biological and genetic patterns of *Lymantria dispar* multiple nucleopolyhedrovirus strain with cubic shape of occlusion bodies

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The strain of *L. dispar* multiple nucleo-polyhedrovirus, characterized by cubic shape of occlusion bodies (OBs) was isolated from a cadaver of *L. dispar* larva collected in Siberia (Asia). After several passages, required for amplification of the virus we estimated both virulence and productivity of the strain. We also sequenced complete genome of "cubic" strain. No cubic shape OBs were found in dead host larvae after the first passage of the strain. After second passage we registered about 30% of cadavers contained cubic shape OBs. OBs were found as the mixture of both typical (polyhedral) and cubic shapes. LC_{50} was 4.6 x 10^6 OBs/ml after the first passage of the strain that was estimated at 12^{th} day for forth instar larvae of *L. dispar*. This result was in agreement with the virulence of typical OBs shape of Siberian strains. However the "cubic" strain productivity of OBs estimated on the fourth instar larvae was very low (6.3 x 10^7 OBs/larvae) in comparison with typical OBs Siberian strains (Akhanaev et al., 2017). Phylogenetic analysis revealed close relation of "cubic" strain to commercial strain of Virin-ENSh biopesticide product. Analysis of nucleotide sequences of both polyhedrin and polyhedral calyx protein genes did not reveal nucleotide polymorphisms between "cubic" and typical strains. The study was supported by Russian Scientific Foundation (grant # 17-46-07002).

Reference: Akhanaev YB, Belousova IA, Ershov NI, Nakai M, Martemyanov VV, Glupov VV (2017). Comparison of tolerance to sunlight between spatially distant and genetically different strains of Lymantria dispar nucleopolyhedrovirus. PLoS ONE 12(12): e0189992.

Poster/Viruses. Wednesday, 13.30. **VR-3**

Development and characterisation of BacMAM vectors for expression of protective genes in pancreatic islet tissue: towards a therapy for Diabetes type 1 in Mexico

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It is estimated that there are more than 35 million patients with type 1 diabetes mellitus (T1DM) worldwide and the number is increasing. T1DM is thought to be caused by auto-immune destruction of the pancreatic islet beta cells that make insulin. Pancreatic islet transplantation is a minimally invasive treatment that has the potential to reverse T1DM leading to improved glycaemic control and abrogating the need for insulin in some patients. One of the current challenges of this procedure however, is trying to prevent the inflammatory and immune destruction of the transplanted islets in a way that would enable this treatment to be used more widely. Pre-transplant gene therapy offers exciting possibilities.

To investigate the use of gene therapy in reducing injury and immune-responses normally associated

with isolation, preservation and transplantation procedures, we developed a set of BacMAM virus vectors. These recombinant baculoviruses encoded an antioxidant gene, superoxide dismutase (SOD-2), an anti-apoptotic gene, B-cell lymphoma-2 (BCL-2) and a reporter gene (eGFP) for monitoring transduction efficiency. To optimise gene expression in human islet tissue, the BacMAM vectors incorporated a high-titre virus backbone and surface display of vesicular stomatitis virus-G (VSV-G). We successfully confirmed the expression of SOD-2, BCL-2 and eGFP in different pancreatic cell lines and donated human pancreatic islets, using fluorescent microscopy and western blotting techniques. These results provide further evidence that the incorporation of the VSV-G protein in a high-titre virus enhance transduction efficiency and confirm BacMAM vectors may be a viable technology to overexpress therapeutic genes in islets. Our long term aim is to apply this improved BacMAM system to increase the success rate of islet transplantation by pre-transplant gene therapy.

Poster/Viruses. Wednesday, 13.30. **VR-4**

Development of BacMAM vectors to improve transduction efficiency of mammalian cells

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Baculoviruses are insect-specific viruses, which have been shown to transduce but not replicate in a wide variety of mammalian cells. So called BacMAM vectors rely on the integration of mammalian promoters within the virus genome to drive expression of the target genes in the designated cell line or tissue.

One drawback of the current BacMAM system is that relatively high multiplicities of infection (100+ virus particles per cell) are vital for effective transduction. This requires either concentration of the BacMAM virus or the use of chemical enhancers of gene expression (e.g. sodium butyrate). Concentration of virus is possible but time-consuming and labour intensive. The use of chemical enhancers is not always desirable due to other effects on cell metabolism, which may be undesirable if the intended use of the BacMAMs is gene therapy.

Here a novel BacMAM virus was constructed that contains a mutation within *fp-25*, which comprises an insertion of an additional "A" that causes a frame shift in the coding region and truncates the native protein. When using baculovirus vectors in insect cells, such mutations are undesirable as they reduce expression from the polyhedrin gene promoter. Conversely, this mutation results in the production of virus stocks with consistent, very high infectious titres, but has no effect on mammalian-specific gene promoters used for expression in BacMAMs. Therefore, transduction experiments using 100+ particles per cell can be achieved without recourse to concentrate the recombinant virus or addition of chemicals to enhance gene expression. The transduction efficiency of this "high-titre" virus backbone was improved further by the incorporation of a truncated vesicular stomatitis virus-G (VSVG) protein into the baculovirus envelope. This most likely increases the uptake of virus into mammalian cells.

These modifications have been tested by transduction of a number of mammalian cell lines with BacMAMs expressing several different genes. They might help in the development of BacMAM vectors as potential candidates for gene therapy, as a desired characteristic for such applications is easy production of high titre stocks to mediate gene delivery.

Poster/Viruses. Wednesday, 13.30. **VR-5-STU**

Display of surface protein by baculovirus for improving the stability of influenza virus hemagglutinin through structure-guided motif swapping

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Hemagglutinin (HA) is the major surface antigen of influenza virus and is the most promising flu vaccine immunogen. However, HA protein instability shortens the shelf-life and may even impair the immunogenicity of influenza vaccines. Here, we applied a novel structure-guided bioinformatics software, SCHEMA, to rationally design chimeric HA, which can be further divided into HA1 and HA2 domains, to improve HA protein stability from the A/Anhui/1/2013 (H7N9) viral strain. This is one of the most fatal influenza viruses threatening birds and humans, however, candidate vaccines against it have exhibited low stability. We used SCHEMA to dissect the structures, and recombine the HA1 subunits from H7 and H3 (A/Aichi/2/1968, H3N2) into chimeric HA1s for thermal stability analysis. Surprisingly, we found that two of the chimeric H7-based HA1s, C2 and C3, displayed much better stabilities than the HA1 of the original H7. We then constructed recombinant baculoviruses to express full-length chimeric HAs by fusing C2 and C3 with HA2 subunit from H7, designated as fC2 and fC3, respectively. Insect cells infected with these recombinant baculoviruses can express the fC2 and fC3 proteins on their cell surfaces, both of which exhibit proper hemagglutination activity. The thermal stabilities of these two full-length chimeric HAs was significantly increased compared with full-length parental H7 protein (fH7), strongly indicating that the swapped domains of these two chimeric

HAs play an important role in the stability of HA protein. Mice immunized with these two chimeric HAs elicited antibodies against fH7. The antisera also successfully inhibited H7N9 infection in a microneutralization assay, suggesting that these two chimeric proteins are better candidate vaccine antigens against H7N9. Our study has significant prospects not only for the generation of more stable H7N9 vaccines, but also providing a novel platform by baculovirus surface display for the functional study of dangerous human-infectious viruses.

Poster/Viruses. Wednesday, 13.30. **VR-6**

Host miRNAs are involved in hormonal regulation of HaSNPV-triggered climbing behavior in *Helicoverpa armigera*

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Baculoviruses manipulate host climbing behavior to ensure that the hosts die at elevated positions on host plants to facilitate virus proliferation and transmission, which is a process referred to as tree-top disease. However, the detailed molecular mechanism underlying tree-top disease has not been elucidated. Using transcriptome analysis we showed that two hormone signals, juvenile hormone (JH) and 20-hydroxyecdysone (20E), are key components involved in HaSNPV-induced tree-top disease in *Helicoverpa armigera* larvae. RNAi-mediated knockdown and exogenous hormone treatment assays demonstrated that 20E inhibits virus-induced tree-top disease, while JH mediates tree-top disease behavior. Knockdown of *BrZ2*, a down-stream signal of JH and 20E, promoted HaSNPV-induced tree-top disease. We also found that two miRNAs target *BrZ2* and are involved in the cross-talk regulation between 20E and JH manipulating HaSNPV replication, time to death and HaSNPV-induced tree-top disease.

Poster/Viruses. Wednesday, 13.30. **VR-7**

Identification of miRNAs and target genes associated with to codling moth resistance against Cydia pomonella granulovirus

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Codling moth (CM, *Cydia pomonella* L.) is an important global pest on pome fruit and a key invasive pest in apple production in China. The Cydia pomonella granulovirus (CpGV, family *Baculoviridae*) has been used as an effective and environment-friendly biological control agent for CM. However, resistance to CpGV products in more than 40 CM populations have been reported. Most of them are against the product of classic type based on the "Mexican isolate" (CpGV-M). This resistance against CpGV-M is dominantly inherited and linked to the sex chromosome Z (Type I resistance). However, the molecular mechanism of type I resistance against CpGV-M is still unclear. Moreover, the reproduction and infection of baculoviruses in many insects are affected by insect microRNA (miRNA) regulation and its target genes. Here, we assessed the miRNAs and their target genes related to CpGV infection and CM resistance. A susceptible and a resistant laboratory CM strains, CpS and CpRR1 (Type I resistance), were used in this study. Small RNA libraries of CpGV-M infected and uninfected CMs of each strain were sequenced. A total of 85 miRNAs and 1,925 their target genes were identified. Thirty-eight miRNAs were found in both infected and uninfected CpS strains, whereas 21 and 11 miRNAs were unique in infected and uninfected CpS strains, respectively. Additionally, infected and uninfected CpRR1 strains shared 33 miRNAs, whereas 23 and 14 specific miRNAs were found in infected and uninfected CpRR1 strains, respectively. Our results suggested that 27 miRNAs were likely related to CpGV infection. Furthermore, 15 genes on Z chromosome were targeted by 18 miRNAs, which were only found in the resistant strain CpRR1, suggesting that these 18 miRNAs may be somehow associated with the resistance of CM to CpGV. Although the verification of those miRNAs and target genes are still ongoing to identify the regulatory roles between candidate miRNA and its target genes, the present study gives a first insight into the potential involvement of miRNA in the CpGV infection and possibly on the molecular mechanism of CM resistance against CpGV.

Poster/Viruses. Wednesday, 13.30. **VR-8**

New method for granulovirus differentiation based on real-time PCR

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Baculoviruses have been used as biopesticides for decades. Recently, due to the excessive use of chemical pesticides there is a need for finding new biological agents that may be useful in crop protection, which are specific, selective and safe for humans, and do not accumulate in the environment or possess high virulence against non-target insects. Sometimes, a few isolates or species are discovered in one host. In the past few years, many new baculovirus species have been isolated from environmental samples, thoroughly characterized and as a result of next generation sequencing (NGS), their genomes are deposited in GenBank database. In general, NGS methodology is the most certain way of detection, but is time-consuming, expensive and qualified staff is necessary to process raw data. Taking into account these limitations, we have searched for faster and more affordable methods for detecting new isolates/species of baculoviruses from the betabaculovirus genus. During our studies, we have developed a method which allows detection and differentiation of new granuloviruses. The method, based on real-time Polymerase Chain Reaction (real-time PCR), allows for distinguishing new granulovirus isolates in only a few hours and at relatively low-cost.

Species from every clade has been chosen on the basis of phylogenetic analysis of betabaculoviruses available in GenBank database. The alignment of highly conserved genes - *granulin* and *lef-9* was performed and the degenerate primers were designed to amplify the most variable, short DNA fragments, flanked with the most conserved sequences. Real-time PCR was then performed, with a melting point curve analysis of each sample. Thereafter, agarose gel electrophoresis was performed with real-time PCR samples. The comparison of melting temperatures allows for rapid and preliminary betabaculovirus species differentiation. In our opinion, the proposed method may be used for screening of new isolates derived from environmental samples.

Poster/Viruses. Wednesday, 13.30. **VR-9-STU**

Novel virus hunting in Australian *tephritid* fruit flies

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Tephritid fruit flies such as Queensland fruit fly (*Bactrocera tryoni*) are major horticultural pests in Australia. Little is known about Australian tephritid pathogens and their potential in biological control as alternative to chemical insecticides that often lack specificity causing damage to beneficial species. Viruses may be effective control agents due to their variable pathogenicity, host specificity, and ability to spread throughout target pest populations. An RNA virus was detected in Sydney laboratory populations of *B. tryoni* over 30 years ago, however it was not further characterised using sequencing approaches. Furthermore, several RNA and DNA viruses are known as pathogenic to relatives, Caribbean fruit fly (*Anastrepha suspensa*), olive fruit fly (*Bactrocera oleae*) and Mediterranean fruit fly (*Ceratitis capitata*) and considered for biological control. Recently, next generation sequencing technology has led to discoveries of novel viruses in *C. capitata*. The viruses have been shown to affect fitness and mortality in fruit fly species, including *B. tryoni*. However, field populations of Australian fruit flies have not previously been tested for viruses and only very limited pathogenicity data exist on the virus that has previously been discovered. Based on available virus sequence information from fruit flies, we have developed RT-PCR to test flies from laboratory reared populations of four species, *B.tryoni,* lesser Queensland fruit fly (*B.neohumeralis*), Jarvis' fruit fly (*B.jarvisi*) and wild tobacco fruit fly (*B.cacuminata*) and flies from wild populations of two species, *B.tryoni* and island fruit fly (*Dirioxia pornia*). The resulting amplified fragments will be identified by Sanger sequencing and compared to known viruses in public data bases. Novel virsues will also be deteced by using a transcriptome analyses. Once a set of viruses of interest have been detected their relative viral load will be quantified by RT-qPCR and used to investigate infection titres across sex, populations and species. This will provide a basis for future research, where the diversity and prevalence of viruses will be tested across populations of *B. tryoni* across climatically different regions of Australia. Future work will also involve extracting the virus from the host and testing it for pathogenicity, transmission and host fitness costs.

Poster/Viruses. Wednesday, 13.30. **VR-10**

Pathogenicity and genome sequence of an isolate of Lymantria dispar multiple nucleopolyhedrovirus from China

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The baculovirus Lymantria dispar multiple nucleopolyhedrovirus (LdMNPV) has been formulatedand applied to suppress outbreaks of the European gypsy moth, *Lymantria dispar dispar*, in the northeastern USA and Canada. Previous studies suggest that the isolate used for *L. dispar dispar* control may be less effective against populations of the Asian gypsy moth (*L. dispar asiatica*), a serious invasive threat to North American forests. As part of ongoing LdMNPV research, an isolate of LdMNPV from a population of *L. dispar asiatica* in Harbin (Heilongjiang province, PRC) was evaluated by laboratory bioassay and genome sequencing. Bioassays were carried out with six different gypsy moth colonies, including *L. dispar asiatica* colonies from populations in China (Harbin, Beijing, and Liaoning province), Japan (Honshu) and South Korea, and an *L. dispar dispar* colony from Connecticut, USA. The Harbin LdMNPV isolate (LdMNPV-Hrb) was tested alongside LdMNPV isolates from Korea and Massachusetts, USA, as well as LdMNPV samples from the commercially available gypsy moth insecticides Gypchek (USA) and Virin-ENSh (former Soviet Union). The bioassays revealed both differences in susceptibilities to LdMNPV among the different colonies and differences in pathogenicity among different isolates. The greatest differences in pathogenicity were observed with the Harbin colony, larvae of which were killed by LdMNPV-Hrb with LC50 values that were 6 – 11X lower than LC_{50} values obtained with the USA isolates. A phylogeny based on whole-genome sequence alignments placed LdMNPV-Hrb in a monophyletic clade with LdMNPV isolates from Japan and Korea. Comparison of LdMNPV-Hrb consensus genome sequences from viruses extracted from Harbin and New Jersey Standard Strain (*L. dispar dispar*) larvae revealed that they differed only by two indels of 57 and 21 bp and a single substitution. These results highlight the value of incorporating additional LdMNPV isolates into LdMNPV-based gypsy moth insecticides, and suggest that these isolates can be produced in an *L. dispar dispar* colony.

Poster/Viruses. Wednesday, 13.30. **VR-11**

Visualization of different protein maturation based on their baculovirus transmembrane signal.

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Baculoviridae is a family of large insect-specific viruses that was broadly studied in last century. Based on extensive research protein expression system have been developed. Baculovirus protein expression system is widely used due to its many advantages such as ease of use, high protein yield and complex posttranslational modifications. Cells in which proteins are produced are easier in culture than mammalian cells, production is cheaper and upscaling is simple. Proteins expressed in insect cells can have various signal sequences directing them into different cell organelle. In our work we have used 2 different signal sequences directing recombinant fluorescent proteins to cell membrane. In one fusion protein we have used transmembrane signal from *Autographa californica nuclear polyhedrosis virus* GP64 which is class III viral fusion protein. In second fusion protein construct transmembrane signal originated from *Lymantria dispar multicapsid nuclear polyhedrosis virus* protein F which belongs to class I of fusion proteins. Moreover the signal sequences were localized differently in both constructs – in one it was located at N and in the other at C-terminus. Transmembrane sequences were fused with fluorescent proteins in one bacmid with separate promoters. Created baculovirus enabled us to observe overproduction, behavior and localization of different constructs during the course of infection.

Poster/Viruses. Wednesday, 13.30. **VR-12-STU**

***Pe38* is not the only gene that responds to type I resistance in codling moth (*Cydia pomonella* L.)**

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Resistant codling moth (*Cydia pomonella* L.) populations have been discovered in more than 40, mainly organic apple plantations in Europe. *C. pomonella* has been listed in the Chinese invasive pest catalogue threatening the major apple production region in China. It is urgent to discover Chinese local CpGV isolates for codling moth control as well as possible occurrence of CpGV resistance in Chinese codling moth populations. Seven new Chinese CpGV isolates (WW, ZY, JQ, ALE, KS1, KS2, ZY2) were field collected in North-West China. After purification and in vivo propagation, bioassays were conducted to determine their virulence to susceptible and CpGV-resistant codling moth larvae expressing type I resistance. The previously reported resistance-breaking marker, a lacking 24-bp repeat sequence in pe38, was screened for by PCR and Sanger sequencing in all isolates. It was found that isolate JQ, KS1 and ZY2 could break type I resistance and isolate ZY showed a typically delayed infection pattern. All isolates followed the pe38 repeat model except WW and KS1. Whereas KS1 contained the 24-bp signature repeat but was resistance-breaking, WW lacked the 24-bp repeat but showed reduced virulence. Therefore, it has to be concluded that additional factor(s) are involved in type I resistance. As a result, it is likely that not only pe38 but (a) further viral gene factor(s) are involved in breaking CpGV type I resistance in codling moth.

Poster/Viruses. Wednesday, 13.30. **VR-13**

Confirmation of two zinc-binding domains in baculovirus protein ME53 and its association with the ribosome during Autographa californica multiple nucleopolyhedrovirus infection

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Baculoviruses are large double-stranded DNA viruses that infect insects of the Lepidopteran order and their uses include biopesticides, recombinant protein expression systems and gene delivery vectors. Autographa californica multiple nucleopolyhedrovirus (AcMNPV), named for the alfalfa looper moth, infects several lepidopteran species. The AcMNPV early/late gene *me53* is required for efficient BV production and is conserved in all alpha and betabaculoviruses. During infection, ME53 is initially localized in the cytoplasm but translocates to the nucleus during late times post infection (12 – 18 hpi) via a highly conserved nuclear translocation sequence (NTS). The 449-amino acid protein also contains two putative C4 zinc finger domains, an N-terminal zinc finger (ZnF-N) and a C-terminal zinc finger (ZnF-C), with the four cysteine residues of each being 100% conserved. C4 zinc fingers are associated with DNA-binding and dimerization domains of nuclear hormone receptors, protein-protein interaction domains, and protein-RNA binding domains. Zinc fingers are classified by their secondary structures which can be inferred by their sequence, however structural studies are required to confirm zinc binding ability and secondary structure. One purpose of this study was to confirm or refute the binding of the two ME53 zinc finger domains with zinc as well as to determine their role in virus infection. Preliminary growth curves show that ZnF-C deletion results in a delay of BV production from 12 to 18 hours post transfection (hpt). Since ME53 is localized in the cytoplasm at early times post infection, the delay suggests that the ZnF-C has a cytoplasmic role. Cytoplasmic functions at early times post-transfection may include translational regulation, which is supported by yeast-2-hybrid data showing that ME53 interacts with the host 40S ribosomal subunit protein RACK1. In this preliminary study we demonstrate by circular dichroism that peptides representing ZnF-N and ZnF-C bind zinc and that ME53 associates with the monosomes and polysomes of virus infected cells.

Poster/Viruses. Wednesday, 13.30. **VR-14**

Interaction of VP80 and ME53 from Autographa californica nucleopolyhedrovirus

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ME53 is a 449 aa structural protein of unknown function with two zinc fingers and is conserved in all sequenced alpha- and betabaculovirus genomes. For the baculovirus Autographa californica nucleopolyhedrovirus (AcMNPV) ME53 is required for efficient virus production and is a structural protein of the nucleocapsid of budded and occlusion-derived viruses. At early times, ME53 localizes in the cytoplasm and, with GP64, forms foci at the cytoplasmic membrane. At late times and despite the absence of a recognizable NLS, ME53 translocates to the nucleus and localizes mainly at the ring zone where occlusion-derived viruses form. AcMNPV-infected or uninfected Sf9 cells were used to construct prey cDNA libraries for use in yeast two hybrid to identify potential protein-binding partners of ME53 using ME53 as bait. Cellular receptor for activated protein kinase C (RACK1) was identified as one putative ME53 protein interacting partner from a cDNA library constructed from uninfected cells. Viral VP80 and LEF5 were also determined as putative ME53 protein binding partners from the cDNA library constructed from AcMNPV-infected cells. These three proteins (RACK1, VP80, and LEF5) and several targeted AcMNPV ORFs were tested for protein-protein interaction by reciprocal yeast two-hybrid. The interaction between VP80, a late viral

protein, and ME53 was further confirmed by colocalization studies. Using N terminally fused GFP ME53 to pull down the ME53-binding partners followed by mass spectrometry analysis we identified HSP60 (heat shock protein 60) as another host ME53-binding protein. Since VP80 is a nucleocapsid component and ME53 localizes at the cellular membrane, interaction between VP80 and ME53 may facilitate the attachment of the nucleocapsids to the cellular membrane to support efficient budding during infection. To better understand the interaction between VP80 and ME53, knockout bacmids were constructed and the effect of these proteins on each other were observed. ME53 fails to localize to the nucleus in the absence of VP80, while nuclear localization of VP80 is not affected in the absence of ME53 suggesting that VP80 may act as a chaperon to transport ME53 into the nucleus late in infection.

Poster/Viruses. Wednesday, 13.30. **VR-15**

Advances in the use of *CRISPR/Cas* technology to edit baculovirus genomes

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Baculoviruses are pathogens of lepidopteran, hymenopteran and dipteran species carrying large double-stranded DNA genomes. They are classified into four genera: *Alphabaculovirus*; *Betabaculovirus*; *Gammabaculovirus*; and *Deltabaculovirus*. The biotechnological importance of these viruses is due to their applications, which include biological pest control, recombinant protein expression technologies and gene therapy. In this sense, it is an important scientific issue to increase the knowledge about them and optimize their genomes according to their uses. In recent years, a new tool originated from bacterial systems and known as CRISPR/Cas emerged to facilitate genome editing. CRISPR/Cas makes it possible to carry out Double Strand Breaks in any DNA sequence, which added to cellular homologous and non-homologous recombination processes made it possible to facilitate genome mutagenesis. According to this, we evaluated *in vivo* (in bacteria) and *in vitro* procedures based on CRISPR/Cas technology to convert baculoviral genomes into modifiable bacmids that multiply in *Escherichia coli*, and then to edit those bacmids to remove or add genetic information. The methodology fine tuning and the conceptual tests were carried out on genetic constructs containing segments of the genomes of AcMNPV and AgMNPV, suggesting that the CRISPR/Cas technology could be applicable in the future as a useful tool in the basic and applied studies on baculoviruses.

Poster/Viruses. Wednesday, 13.30. **VR-16**

AcMNPV-miR-2 facilitates AcMNPV infection by down-regulating the expression of viral own genes and host immune related proteins

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MicroRNAs (miRNAs) play diverse regulatory roles in broad biological processes. In the present study, we show that AcMNPV-miR-2, an Autographa californica Nucleopolyhedrovirus (AcMNPV) encoded miRNA, expresses during early stage of infection, and negatively regulates the expression of viral late expression factors (*lef-6*, *lef-11*) and other early genes, is vital for the early stage of viral infection in Sf9 cells. We explored the effects of AcMNPV-miR-2 on virus infection in Sf9 cells. The results showed that AcMNPV-miR-2 overexpression decreased the production of infectious budded virions (BVs), reduced viral DNA replication, and delayed the death of *Trichoplusia ni* larvae. Besides, an obviously lagging in the polyhedron formation was observed by light microscopy and transmission electron microscopy (TEM) upon overexpression of AcMNPV-miR-2, it may be due to AcMNPV-miR-2 down-regulated the expression of polyhedrin and p10. Furthermore, we assessed the host immune response by detecting the expression of the *Lepidoptera*-specific immune related proteins, gloverin and spod-11-tox upon overexpression or inhibition of AcMNPV-miR-2. Our findings showed that the expression of the two immune related proteins was remarkably reduced and increased with the overexpression and inhibition of AcMNPV-miR-2, respectively. It implies that AcMNPV-miR-2 can help AcMNPV to counter host immune responses. All these results suggest that AcMNPV-miR-2 can facilitate viral infection through down-regulating the expression of viral own genes and host immune related proteins.

Poster/Viruses. Wednesday, 13.30. **VR-17**

Characterization of *Autographa californica Nucleopolyhedrovirus ac75* and its role in the morphogenesis of budded virions and occlusion-derived virions

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During the baculovirus life cycle, the morphogenesis of both budded virions (BVs) and occlusion-derived virions (ODVs) is proposed to involve a budding process at the nuclear membrane, which occurs while nucleocapsids egress from the nucleus or when intranuclear microvesicles are produced.

However, the exact mechanism of virion morphogenesis remains unknown. In this study, we identified Autographa californica nucleopolyhedrovirus (AcMNPV) *orf75* (*ac75*) as a second gene, in addition to *ac93*, that is essential for the nuclear egress of nucleocapsids and intranuclear microvesicle formation. *ac75* is a highly conserved gene of unknown function. We constructed an *ac75* knockout AcMNPV bacmid and investigated the role of *ac75* in the baculovirus life cycle. The expression and distribution of the Ac75 protein were characterized, and its interaction with another viral protein was analyzed to further understand its function. The knockout of *ac75* blocked the generation of BVs. Electron microscopy indicated that *ac75* was required for the egress of nucleocapsids from the nucleus and for the formation of intranuclear microvesicles, which are precursor structures of ODV envelopes. Western blot analyses showed that two forms, of 18 and 15 kDa, of FLAG-tagged Ac75 protein were detected. Ac75 was associated with both nucleocapsid and envelope fractions of BVs, but only the nucleocapsid fraction of ODVs; the 18-kDa form was associated with only BVs, whereas the 15-kDa form was associated with both types of virion. A phase-separation assay suggested that Ac75 was not an integral membrane protein. However, it interacts with an integral membrane protein (Ac76) and is associated with the nuclear membrane. These data enhance our understanding of the commonalities between nuclear egress of nucleocapsids and intranuclear microvesicle formation and may help to reveal insights into the mechanism of baculovirus virion morphogenesis.

Poster/Viruses. Wednesday, 13.30. **VR-18**

Determination of *Anticarsia* MNPV and *Spodoptera* MNPV co-infection in insect cell culture

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The velvetbean caterpillar (*Anticarsia gemmatalis*) is an important pest for soybean, especially in South Brazil. The fall armyworm (*Spodoptera frugiperda*) which is the most important maize Lepidoptera pest in this country, has been lately reported to also attack soybean in areas where these crops are used in succession. Larvae of both species can be controlled by *Spodoptera frugiperda MNPV* (SfMNPV) and *Anticarsia gemmatalis MNPV* (AgMNPV), respectively. Baculovirus *in vitro* production is an interesting approach to overcome some difficulties of *in vivo* production. Co-infection of a host cell with two distinct viruses can result in co-occlusion phenotype and this feature might be important to reduce final costs. In this work the susceptibility of IPLB-SF-21AE cell line to a mixed infection of SfMNPV and AgMNPV was tested. At first, cells were seeded at a density of $1X10^6$ per $60mm^2$ dish. The initial co-infection (P0) was done with Budded Viruses (BVs) previously obtained from SfMNPV-I9 and AgMNPV-2D isolates. Different proportions of SfMNPV/AgMNPV (µl of inoculum) were tested (100:100, 10:100, 100:10) and infected cells were kept in TNMFH complete medium at 27^0C. Morphological changes were monitored by light microscopy during five days. Then, the supernatants were collected for new infections (P1) using the same procedure. At 5 d.p.i. the supernatants were collected for the second passage (P2). The amount of DNA obtained from BVs and from polyhedra of the three passages was monitored by real-time PCR (qPCR) using Sybr green system with primers designed to *gp64* of AgMNPV and *sf32* of SfMNPV. Besides, the kinetics of viral protein synthesis was carried out for analysis of the co-infection in P0 passage (100:100 inoculum). The results demonstrated successful co-infection in these cells. The amount of DNA from SfMNPV and AgMNPV in supernatants and sediments tends to be maintained stable during the three passages, although the amount of AgMNPV is higher than SfMPNV in the majority of the experiments. Analysis of the kinects of radiolabed proteins showed that the cell protein synthesis was shut off and two distinct bands of about 30 kD, regarded to be the polyhedrin of each virus, were strongly detected at 48h.pi. Support:FAP-DF.

Poster/Viruses. Wednesday, 13.30. **VR-19**

Functional studies on the host AAA+ ATPase Ter94 in baculovirus life cycle

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Ter94 is a member of AAA+ (ATPases associated with diverse cellular activities) ATPase family, which utilize the energy of ATP hydrolysis to remodel substrates. It is believed that Ter94 plays important roles in diverse cellular processes including membrane fusion, cell cycle control, ER-associated protein degradation (ERAD), mitochondria associated degradation (MAD), autophagy, *etc.* Many viruses recruit Ter94 to assist virus replication. However, little is known about the function of Ter94 in baculovirus life cycle. In this study, we analyzed the expression and localization of Ter94 in Autographacalifornica multiple nucleopolyhedrovirus (AcMNPV) infected cells. Results showed that Ter94 was accumulated in the

ring zone upon baculovirus infection. Immunoelectron microscopy and Western blot analyses showed that Ter94 was associated with nucleocapsids of both BVs and ODVs. By using RNAi and inhibitor treatment, Ter94 was found to be involved in AcMNPV genome replication, nucleocapsid egress from nucleus to cytoplasm, as well as ODV envelopment within nucleus. Viral proteins that interact with Ter94 were sophisticatedly exploited. These data revealed that host ATPase Ter94 plays a crucial role in baculovirus infection and morphogenesis. Results implied that energy supply were necessary during viral life cycle.

Poster/Viruses. Wednesday, 13.30. **VR-20**

The conserved amino acid N27 of baculovirus Ac110 is important for oral infection

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The family Baculoviridae is a highly selective pathogen in arthropods, mainly in insects of the order Lepidoptera. Ac110 is one of the *per os infectivity factors* that are essential for the initial infection of larvae in midgut cells, but the mechanism by which Ac110 employs to affect the midgut infection is still unknown. In the present study, two recombinant viruses, vAc110N27A and vAc110L35A were generated in which the conserved amino acids N27 and L35 of Ac110 were mutated to alanine, respectively. The expression of the mutant proteins was confirmed by Western blot analysis. Transfection and infection assays and viral growth curves analysis were done by using Sf9 cells, the results showed that both the recombinant viruses were able to produce compatible infectious budded viruses and the spread of the viruses among Sf9 cells was not affected compared with the control virus. Bioassays were performed by feeding *Spodoptera exigua* larvae with purified polyhedra to detect the ability to initiate midgut infection for both recombinant viruses. The mortality rate of vAc110N27A was significantly reduced compared to the control virus, while no difference was observed for vAc110L35A. Thus we conclude that N27 of Ac110 is important for the function of Ac110 during *per os* infection.

Poster/Viruses. Wednesday, 13.30. **VR-21**

Construction of Baculovirus inducible expression system for rapid development of virus like particle vaccines

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Virus-like particles (VLPs) are similar to pathogenic viruses, but do not have nucleic acids, so they have higher safety and immunogenicity than other vaccines. However, in the selection of various structural proteins to form VLPs, all expression systems consume a large amount of time in common. Among them, the baculovirus expression system causes additional time consumption to construct the recombinant baculovirus. Therefore, there is a need for a system that can rapidly determine the structural proteins required for effective VLP production. This study aims to solve this problem by constructing a virus inducible expression platform through the production of efficient baculoviruese and inducible vectors. The platform was evaluated for overexpression using EGFP. We also confirmed the formation of virus like particles through overexpression of virus structural proteins.

Contributed papers Pipeline

Wednesday 16.00-18.00

BACTERIA 2

Moderator: O. P. Perera

Contributed paper Wednesday 16.00 **102**

Alkaline adaptation of *Bacillus thuringiensis* regulated by Crp protein

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As an entomopathogen, *Bacillus thuringiensis* (Bt) have to survive in the midgut environment of the target insect. Lepidoptera larval midgut has alkaline environment. This study mainly explores effect of L-lactate metabolism on alkaline adaptation of Bt. Bt HD73 strain stopped growing in the beginning when alkali was added to the medium with a final concentration of 28 mM, while the bacteria can restart growth at about pH 9. The higher concentration of NaOH being added, the longer period was needed for the restored growth. Microarray data indicated that the transcription of ldh2 (encoding Lactate dehydrogenase) is up-regulated significantly under alkaline condition. Analysis of promoter activity by lacZ fusion constructions also confirmed this. Under alkaline condition, secretion of L-lactate increased significantly. cAMP receptor protein (Crp) is an important transcriptional factor in prokaryotic cells. crp genes are up-regulated under alkaline condition, and Crp binding site was found in the promoter region of ldh2. Electrophoretic mobility shift assays indicated that cAMP-Crp complex can directly bind to the ldh2 promoter region in vitro. Compared to the wild strain, transcriptional activity of ldh2 was significantly decreased in crp mutant. Either ldh2 or crp gene deletion mutant restarted growth slower than the wild strain. L-lactate secretion in the mutant also decreased under alkaline condition. These result suggest that L-lactate give contribution to the alkaline adaptation of Bt, and the metabolism is regulated by transcriptional factor Crp.

Contributed paper Wednesday 16.15 **103-STU**

Characterization of the resistance to *Bacillus thuringiensis* Vip3Aa protein in a *Heliothis virescens* laboratory population

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Genetically modified crops expressing Cry and Vip proteins from *Bacillus thuringiensis* are used to control different pests, though the long-term use of these crops is threatened by the occurrence of resistance. Here, two *Heliothis virescens* populations were analysed at the biochemical and genetic level, one susceptible (UNSEL) and another resistant (SEL) to Vip3Aa protein. Enzymatic assays showed a significant decrease of alkaline phosphatase (ALP) activity in the SEL strain. Ligand Blotting and binding assays with radiolabelled Vip3Aa demonstrated no binding site alteration, suggesting that other mechanisms can be responsible for resistance. A previous study showed an association between altered gene expression levels of ALP and several ATP-Binding Cassette transporter subfamily C (ABCC) members with Cry1-resistance. Based on these findings, the expression levels of these genes were explored in both strains. For this purpose, data mining and de novo assembly were performed, describing the presence of two new ABCC genes in *H. virescens*. RT-qPCR showed significant downregulation on the ALP (10-fold) and ABCC (from 6 to 1000-fold) genes. Understanding the basis of the resistance is an effective way to help delay the evolution of resistance in Bt crops.

Contributed paper Wednesday 16.30 **104**

Defining the virulence determinants of *Serratia proteamaculans* AGR96X and its capacity for protection of establishing ryegrass from larvae of New Zealand grass grub (*Costelytra giveni*) and manuka beetle (*Pyronota* spp.)

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A highly virulent *Serratia proteamaculans* strain, AGR96X, exhibiting specific pathogenicity against larvae of the New Zealand grass grub (*Costelytra giveni*; Coleoptera: Scarabaeidae) and the New Zealand manuka beetle (*Pyronota festiva* and *P. setosa*; Coleoptera: Scarabaeidae), was isolated from a diseased grass grub larva. Unlike the grass grub specific *Serratia entomophila* which takes 1-3 months to kill the host, AGR96X invades the hemoceol within 3-5 days of ingestion where the bacterium rapidly multiplies to a high cell density causing death of the target insect within 5–12 days of ingestion. The main virulence determinant of AGR96X is a variant of the *S. entomophila* anti-

feeding prophage (Afp), a phage-like tailocin designated AfpX. Unlike the Afp, AfpX contains two Afp16 tail-length termination protein orthologues and two putative toxin components. Transmission electron microscopy analysis revealed the presence of Afp-like particles of varying lengths, and when the purified AfpX tailocin was fed to grass grub or manuka beetle larvae, they underwent similar phenotypic changes to larvae fed AGR96X. Pot trials assessing *S. proteamaculans* AGR96X applied as a seed coat found the bacterium to be effective at controlling larvae of both grass grub and manuka beetle, giving comparable, if not greater protection than chemical insecticides. The rapid lethality and broader host range of AGR96X make this bacterium a viable alternative to *S. entomophila* for pest control.

Contributed paper Wednesday 16.45 **105**

Two strong promoters for cry gene expression

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Here, we report a novel σ^E-dependent strong promoter of a non-cry gene (HD73_5014) from *B. thuringiensis* subsp. *kurstaki* HD73 (*B. thuringiensis* HD73), which can direct strong cry1Ac gene expression in *B. thuringiensis* HD73. We constructed an *E. coli-B. thuringiensis* shuttle vector (pHT315-P5014-1Ac) for cry1Ac gene expression, using the HD73_5014 gene promoter. SDS-PAGE and western blot analysis showed that expression of the cry1Ac gene directed by the HD73_5014 gene promoter was at the same level as that directed by the previously known strongest cry promoter, Porf1-cry8E. The strain with Cry1Ac protein expression under the control of the HD73_5014 gene promoter (P5014-cry1Ac) showed insecticidal activity against *Plutella xylostella* similar to that under the control of the *cry*8E gene promoter (*Porf*1-*cry*8E-*cry*1Ac). We also tested the activities of both P5014 and *Porf*-*cry*8E promoters in spoIIID and sigK mutants. The results showed that they had a similar activity in these backgrounds. Collectively, these results suggest that the HD73_5014 gene promoter, as a non-*cry* gene promoter, and *Porf*1-*cry*8E would be the efficient transcriptional elements for cry gene expression. These data also show the possibility for improving Cry production by searching for transcriptional elements in not only cry genes, but also non-*cry* genes.

Contributed paper Wednesday 17.00 **106**

Bt or not Bt? Genome analysis of mosquitocidal *Bacillus wiedmannii* biovar *thuringiensis* strain FCC41

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Bacillus cereus sensu lato also known as *Bacillus cereus* group is composed of an ecologically diverse bacterial group with an increasing number of related species, some of which are medically or agriculturally important. Numerous efforts have been undertaken to allow presumptive differentiation of B. cereus group species from one another. FCC41 is a Bacillus cereus group strain toxic against mosquito species like *Aedes aegypti*, *Aedes* (*Ochlerotatus*) *albifasciatus, Culex pipiens, Culex quinquefasciatus* and *Culex apicinus*, some of them responsible for the transmission of vector-borne diseases. Here, we report the complete genome sequence of FCC41 strain, which consists of one circular chromosome and eight circular plasmids ranging in size from 8 to 490 kb. This strain harbors six crystal protein genes, including cry24Ca, two cry4-like and two cry52-like, a parasporin gene cry41-like and multiple virulence factors. The phylogenetic analysis of the whole-genome sequence of this strain with molecular approaches such as ANI, MLST, core genome genes, rpoB and panC places this strain into *Bacillus wiedmannii* cluster. However, according with phenotypical characteristics such as the mosquitocidal activity due to the expression of some cry genes found in a parasporal body and encoded in plasmids of different sizes, this strain could be renamed as *B. wiedmannii* biovar *thuringiensis* strain FCC41.

Contributed paper Wednesday 17.15 **107**

Examining putative insecticidal toxins from the bacterium *Brevibacillus laterosporus*

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In this study we attempted to identify the main toxins produced by two New Zealand strains of *Brevibacillus laterosporu*s, Bl 1951 and 1821, with known activity against the diamondback moth (DBM), *Plutella xylostella.* Genome sequencing revealed the presence of cry genes in both strains, which were hypothesised to be the cause of caterpillar toxicity. Transmission electron

microscopy of sporulating cells showed that Bl 1821 formed large crystals, as would be expected of Cry toxins. *Brevibacillus laterosporus* 1951, despite containing cry encoding regions, does not produce parasporal crystals. The putative larvicidal toxin encoding a *cry*27-like gene from Bl 1821 and 1951 and a *cry*35-like gene from Bl 1951 were heterologously expressed in *Escherichia coli*. The purified and refolded recombinant Cry27-like and Cry35-like proteins did not show any insecticidal activity toward DBM-larvae in bioassays. The Cry-proteins may have mosquitocidal activity, as both Bl 1821 and 1951 have strong larvicidal mosquito activity as well. The Cry35-like protein is likely part of a binary toxin. Further research is now targeting other potential toxins produced by these strains.

Contributed paper Wednesday 17.30 **108**

Cancelled

Contributed papers Maui 3

Wednesday 16.00-18.00

Beneficial Invertebrates & Viruses 2

Moderators: Lyric Bartholomay and Kelly Bateman

Contributed papers Wednesday 16.00 **109**

Histone deacetylase inhibitor-treatment restores memory-related gene expression and learning ability in neonicotinoid-treated *Apis mellifera*

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Apis mellifera (western honeybee) is nature's most essential pollinator for plants. The colony collapse disorder (CCD) had fiercely damaged the population of global honeybees. The main reasons for that cause CCD are associated with the variety of pathogens in bee hive and pesticide-caused memory loss. Histone deacetylase inhibitors (HDACi) are chemical compounds that inhibit the activity of histone deacetylases and are known to cause hyperacetylation of histone cores and influence gene expression. In our previous work, we have demonstrated that HDACi sodium butyrate (NaB) are given to honeybees as a dietary supplement and can up-regulate the expression profiles of immunity and detoxification genes, even for the imidacloprid-treated bees. NaB can also strengthen honeybees' tolerance to imidacloprid and invasions of Nosema ceranae and viral infections. In this study, we focus on NaB effect on learning and memory ability of imidacloprid-treated honeybees. Results showed that the memory-related genes were up-regulated and higher learning ability examined by proboscis extension reflex (PER). We found that HDACi can boost memory formation and learning of bees. This study investigated the association between gene expression and memory formation from an epigenetic perspective and demonstrated the possibility of enhancing bee learning using HDACi.

Contributed papers Wednesday 16.15 **110**

Coral, photosynthesis, and the emergence of parasitism in Apicomplexa

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The discovery over a decade ago that apicomplexan parasites contain a relict plastid organelle prompted many questions about the nature of the parasites' ancestors and the role of photosynthesis and its loss in their evolution. More recently, the discovery of still-photosynthetic relatives of apicomplexa, *Chromera* and *Vitrella*, living in some association with coral has prompted further speculation on the role of photosynthetic symbioses with corals in that evolution. Resolving these questions is confounded by a poor understanding of the real distribution of photosynthesis in apicomplexans and their relatives, and by a poor understanding of the functional relationship between coral-associated apicomplexan relatives and their putative animal "host". Moreover, two key lineages in this story remain known only as "unidentified environmental clades": Genotype N, known from nuclear SSU rRNA, and Apicomplexan-Related Lineage-V, or ARL-V, known from plastid SSU rRNA. Here, we re-evaluate the environmental distribution of coral-associated apicomplexans, and show that *Chromera* and *Vitrella* are not actually associated with coral per se, but rather with coral reef environments more broadly. ARL-V and GenotypeN are truly coral-associated, but co-occurrence patterns in natural samples suggest they are the same organism. This is confirmed by in situ co-localization in lab-cultured corals, which shows both sequences occur in the same cells at the distal edge of the mesenterial filaments of the host coral, altogether suggesting they most likely correspond to a previously described genus of coral apicompelxan, Gemmocystis. Using metagenomic data from coral communities, we assembled near-complete plastid genomes from samples positive for ARL-V plastid SSU rRNA, confirming ARL-V to be non-photosynthetic, but interestingly retaining genes involved in chlorophyll biosynthesis.

Contributed paper Wednesday 16.30 **111-STU**

Cracking the code of Pacific oyster mortality syndrome

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Infectious diseases are very often explored using reductionist approaches, despite repeated evidence showing them to be strongly influenced by numerous interacting host and environmental factors. Many diseases with complex etiology therefore remain misunderstood. By developing a holistic approach to tackle the complexity of the interaction, we deciphered the complex intra-host interactions underlying the Pacific oyster mortality syndrome affecting juveniles of *Crassostrea gigas*, the main oyster species exploited worldwide. Using ecologically realistic experimental infections combined with thorough molecular (metabarcoding, transcriptomics, pathogen monitoring) and histological analyses on oyster families with contrasting susceptibilities, we demonstrated that the disease is caused by a multiple infection whose initial and necessary step is the infection of oyster hemocytes by a herpesvirus. Viral replication leads to an immune-compromised state of the host, evolving toward subsequent bacteremia by opportunistic bacteria. By identifying critical intra-host interactions between microorganisms and host immunity, this study cracks the code of the Pacific oyster mortality syndrome and provides important molecular data for the design of prophylactic measures and breeding programs dedicated to the production of oysters resistant to the mortality syndrome. We believe that such a systems biology approach could be applied to decipher other multi-factorial diseases that affect non-model invertebrate species worldwide.

Contributed papers Wednesday 16.45 **112**

Honey Bees in Peril: The use of cricket paralysis virus as a model honey bee virus system to study colony collapse disorder

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Honey bees (*Apis mellifera*) play a vital role in pollinating both agricultural crops and wild plants. However, over the past several decades honey bee populations have been declining in a phenomenon known as Colony Collapse Disorder (CCD). The ectoparasitic mite, *Varroa destructor,* has been a large contributor to CCD, as it is known to transfer viruses that may ultimately lead to colony loss. Previous research has shown varroa mites enhance the effects of these infections as well, causing abnormalities in bee physiology and behavior. Looking specifically at physiology, our project analyzed how a dicistrovirus, cricket paralysis virus (CrPV), affects the gene expression of vitellogenin. Vitellogenin is linked with regulatory behaviors; low amounts promote frantic foraging behaviors that may aid in CCD. Viremia was quantified using $TCID_{50}$ assays between various treatment groups (CrPV, CrPV+ *V. destructor* protein extract, and PBS). An optimal procedure for multiplex quantitative reverse transcription PCR (RT-qPCR) using One-Step iTaq Probes was then developed. Probes were designed to target vitellogenin mRNA, CrPV viral RNA, and β-actin (reference gene). Results showed honey bees injected with *V. destructor* protein extract + CrPV had a dramatically higher levels of viremia compared to CrPV-injected bees only, according to the $TCID_{50}$ results. PBS-injected bees exhibited no viremia. Relative vitellogenin expression was significantly lower in the *V. destructor* protein extract+CrPV and CrPV infected bees compared to control bees. These results uncover a potentially substantial physiological relationship between viral disease and vitellogenin that may inform us about some of the underlying mechanisms of colony collapse disorder.

Contributed paper Wednesday 17.00 **113**

Divergence from the PDV paradigm in the repeated evolution of associations between mutualistic viruses and parasitoid wasps

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Some lineages of parasitoid wasps have evolved a remarkable strategy in their parasitism arsenal:

they utilize symbiotic viruses to breach host defenses. The most well-known examples are Polydnaviruses (PDVs), which are found in three exceptionally diverse clades of parasitoid wasps and represent three unique origins. Despite their independent evolution, PDV genomes share some key characteristics. PDV genomes are integrated into the genomes of wasps, and their genome architecture has been rearranged compared to viral ancestors into two separate components: proviral segments containing virulence genes and replication genes. Both replication genes and proviral segments are located singly or in clusters spread throughout wasp genomes. The replication machinery for these viruses is not packaged into virions, thus the viruses and parasitoids are reliant on each other for reproduction. In addition to PDVs, symbiotic viruses have been documented in other parasitoid wasp species, and their recent genomic characterization has shown that these virus symbionts diverge from the PDV paradigm. The genome sequence of the entomopoxvirus found in *Diachasmimorpha longicaudata* venom glands (DlEPV) revealed that this virus is non-integrated and can replicate in host insects. Although the DlEPV genome differs in architecture relative to PDVs, viral transcriptome analysis shows that virulence and replication genes are partitioned in wasps and hosts at the level of expression, functionally behaving in a PDV-like manner. We have discovered another independently-derived viral symbiont in *Fopius arisanus* that has a wasp-integrated genome that produces virus-like particles that do not contain nucleic acids. Despite a very young association with wasps in the genus *Fopius*, rearrangement of genome architecture has already occurred for this virus symbiont, highlighting the adaptive advantage of this process. These data highlight the diversity of viral symbiosis strategies and variation in genome architecture, which has important implications for symbiont function in hosts.

Contributed papers Maui 1 & 2

Wednesday 16.00-18.00

Fungi 2

Moderator: Komivi Akutse

Contributed paper Wednesday 16.00 **114**

Germination of *Beauveria pseudobassiana* microsclerotia and biocontrol activity against the African black beetle (*Heteronychus arator*)

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A New Zealand isolate of *Beauveria pseudobassiana* obtained from the African black beetle (*Heteronychus arator* F.) was previously described as forming microsclerotia (MS), which are melanized resting structures, which are able to survive for long time periods in soil. MS were produced in liquid fermentation and after 15 days, were harvested and air-dried. The germination process was studied after placing dry MS on the surface of water-agar plates and soil. Samples taken from the agar surface after incubation at 25°C for two and four days were processed and observed with scanning (SEM) and transmission (TEM) electron microscopes. SEM images showed MS with irregular shape and rough surface. Surface showed abundant hyphal growth after two days and phialide and conidia formation after four days. TEM images showed lack of MS membranous or cellular cover and also showed an intercellular matrix surrounding cells in division phase during germination. On the other hand, membranous structures similar to lomasomes inside the cells were observed. Four days post germination, dead cells were apparently more frequent than two days post germination. Conidia produced by germinated MS were harvested after seven days and evaluated in a laboratory bioassay against African black beetle adults. Insect mortality reached 45% and cadavers presented typical symptoms of fungal infection. MS germination and sporulation on soil with two levels of moisture were also evaluated and both variables were directly correlated with soil moisture. MS germination (48h) was 80% and 100% for soil at 17% and 30% moisture and after seven days, *B. pseudobassiana* concentrations in soil were 104 and 105 CFU/g of soil, respectively. *B. pseudobassiana* MS demonstrated the potential to be used as a new sustained release strategy of conidia for control of important soil-dwelling insects such as *H. arator.*

Contributed paper Wednesday 16.15 **115**

Leptoglossus occidentalis (Heidemann, 1910), an invasive species attacking conifers in Lebanon: preliminary laboratory control by the entomopathogen *Beauveria bassiana*

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The western conifer seed bug, *Leptoglossus occidentalis* (Heidemann, 1910), is an alien invasive species originating in North America and attacking conifers. The first record of the pest in Lebanon was in 2015. It got established in different pine forest regions from North to South of Lebanon and was associated with dry cone syndromes. The objective of the study was to evaluate the efficacy of a biological control agent, *Beauveria bassiana*, on the immature stages of *L. occidentalis* under laboratory conditions. Chemical control of this pest is problematic because of the ecology of the insect and the restriction of chemical pesticides uses in forest ecosystems. The entomopathogenic fungus *Beauveria bassiana*, a potential biological control against immature stages of *L. occidentalis* was evaluated under laboratory conditions. Two concentrations of conidial suspension were applied topically on eggs and first nymphal instars: 50 conidia/immature and 500 conidia/immature. The hatching of eggs treated with conidial suspension was 40% lower compared to 100% in the control 100%. *B. bassiana* caused 100% mortality of first instar and sporulation of the insects' cadavers occurred after 3 weeks. The present study shows that *B. bassiana* is a potential biocontrol agent to reduce the population of the alien insect.

Contributed paper Wednesday 16.30 **116**

Wheat growth-response to endophytic *Beauveria bassiana* following fungal encounters from insect versus plant sources

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Recovery of the entomopathogenic fungus (EPF), *Beauveria bassiana* (Hypocreales: Cordycipitaceae) from soil (via insect cadavers and/or selective media) and the phylloplanes of natural and crop vegetation, suggests an ubiquitous presence within agroecosystems. However, proliferation and long-term survival relies on recycling, either as etiological agent in an insect host or as saprophyte from plant organic matter (including an endophytic pathway towards colonising such material). Essentially, the agroecosystem may thus, harbor an insect- and/or plant-derived source of *B. bassiana* inoculum. This study was aimed at quantifying the plant growth response in wheat, *Triticum aestivum*, following fungal encounters with these two sources. *Beauveria bassiana*, strain PPRI 7598, was passed through the Russian wheat aphid (RWA), *Diuraphis noxia*, and *T. aestivum*, to establish an insect (IN) and plant (PL) 'background'. When five cultivars were inoculated through seed imbibition with the two 'backgrounds', a significantly higher mean level of endophytic colonisation (roots, stems and leaves, combined) was recorded with 7598IN (29.74%) compared to 7598PL (26.13%). A significant cultivar effect was also noted, with Baviaans rated as the most colonised cultivar (plant parts pooled = 33.54%) followed by Tugela (31.34%), Kariega (27.87%), Gariep (25.67%) and Elands (21.28%). Seven days post inoculation, the highest levels of colonisation were 44.15% and 38.59% (cultivars pooled), recorded in roots with 7598IN and 7598PL, respectively. Growth response to three inoculation techniques, i.e., seed imbibition, soil drenching and leaf spraying, showed leaf spraying to be the most effective method. Endophytism was particularly beneficial in promoting fresh root biomass, especially in cultivar Kariega, which showed a 260% increase cover controls. Overall, *B. bassiana*-treated plants responded positively, exceeding control growth parameters (pooled) by an average of 71%. In assays against RWA, 7598IN caused 57% mortality, compared to 50% by 7598PL. 7598IN showed a significantly shorter mean time of mortality (4.14 days) compared to 7598PL at 4.58 days, and the level of overt mycosis recorded with 7598IN (58.2%) was significantly higher compared to 7598PL (47.9%). Through this study, endophytic colonisation of five wheat cultivars by *B. bassiana* 7598 was confirmed. Generally, the insect-derived fungal source outperformed the plant-derived source in both endophytic and insect-pathogenic parameters measured.

Contributed paper Wednesday 16.45 **117-STU**

Elucidating the natural function of cordycepin, a metabolite of the fungus *Cordyceps militaris*

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Cordyceps militaris (Ascomycetes, Hypocreales) is a teleomorphic entomopathogenic fungus (EPF) that produces fruiting bodies on naturally infected lepidopteran pupae in the soil. It synthesizes the secondary metabolite, nucleoside analogue cordycepin (3'-deoxyadenosine). *C. militaris* is used as traditional medicine, alongside *Ophiocordyceps sinensis* (which also produces cordycepin). Cordycepin is currently being investigated as an anti-inflammatory and anti-cancer drug in human medicine, which includes mode of action studies. However, very little is known about its natural function, i.e. in fungal-infected insects. The aim of this work is to better understand the natural function of cordycepin, including research on its effect on the insect immune system, with the long term goal of using cordycepin as a potential biopesticide. A series of bioassays and qRT-PCR analyses have been carried out in *Galleria mellonella* larvae and *Drosophila melanogaster* S2 cells to quantify the effects of cordycepin on the

expression of insect immune-related genes when applied alone and in combination with EPF. Application of cordycepin is able to inhibit the immune response by reducing the expression of anti-microbial peptides (AMPs). This suggests that cordycepin may have a role in facilitating the infection of insects in nature. Bioassays to quantify the effect of foliar-applications of cordycepin against diamondback moth Plutella xylostella have shown both antifeedant and host mortality effects.

Contributed paper Wednesday 17.00 **118**

Combined utilization of *Beauveria bassiana* and spinosad against wireworms *Agriotes lineatus* and *Agriotes obscurus* (Coleoptera: Elateridae)

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The complex of click beetle larvae (Elateridae), known as wireworms, are among the most economically damaging pests for the maize and potato producers in all climatic zones where these crops are cultivated. As Governments' environmental policies are typically resulting in older and more persistent insecticides being withdrawn from the market, 'biorational' alternatives are urgently being sought to replace them. *Beauveria bassiana* (Bals.-Criv) Vuill. is an effective entomopathogenic fungi against a broad-spectrum of pests and a number of strains have been registered and commercialized around the world. This work aimed to establish whether or not *B. bassiana*, either alone or as part of an IPM programme, could deliver effective management of wireworm. The susceptibility of wireworm to a commercial strain of *B. bassiana* was evaluated both on its own and as part of a stress and kill strategy in combination with a sublethal dose of spinosad. The Spinosad was applied as soil drench at concentration of 1.6ppm, 3.2ppm, 6.4ppm and 12.8ppm active ingredient per gram sand, with or without a constant concentration of *B. bassiana* of $1*10^6$ conidia per gram sand was applied. After 6 weeks no synergy was noted between Beauveria and the spinosad. Spinosad alone at 6.4ppm and higher was enough to reduce the feeding activity and increased the number of moribund wireworm, but no mortality was observed in any of the treatments. An increase of feeding activity was noted in presence of the *B. bassiana*, which was possibly due to artefacts of the formulation, such as CO_2 or other co-formulants. Future work will aim to identify more effective strains of entomopathogen and alternatives active ingredients which may help with synergizing the effects of the pathogens.

Contributed paper Wednesday 17.15 **119**

Multigene systematics and ecological associations of two proposed new species of Metarhizium from Australia.

S. M. N. Islam, T. Scharaschkin & C. Hauxwell

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Hypocrealean fungi were isolated from agricultural fields, grassland and forest soils at three locations in Queensland, Australia. A total of 164 isolates were identified as belonging to the genus *Metarhizium* based on their appearance on selective medium and ITS sequence. A multi-locus analysis of concatenated data sets of MzIGS3 and 5'-TEF inferred the taxonomic position of these isolates in five well-supported monophyletic clades. Three of the clades were assigned to known species: *M. robertsii, M. pingshaense* and *M. anisopliae*. Two other clades were strongly differentiated and represent two proposed new *Metarhizium* species in a complex with *M. pingshaense.* Fungal species were significantly associated with specific crops, ecotypes and soil components: *M. anisopliae* and *M. robertsii* were associated with soil from legume crops and forests, and with higher nitrogen and carbon content in soil, whereas the new species were associated with maize and grassland and with soils of lower nitrogen and carbon content. This supports the potential to increase the success of *Metarhizium spp.* as agricultural inocula by selection of fungal isolates.

THURSDAY 16 August 2018

Fungal Division Symposium

08.00-10.00 Pipeline

Fungus-insect interactions in post genomic era: Advances and perspectives - Genomic and transcriptomic studies on *Beauveria* including plant associations

Organisers/Moderators: Chengshu Wang and Jae Su Kim

Symposium Thursday 8.00 **120**

Genomic and transcriptomic studies on *Beauveria* including plant associations

Travis R. Glare[1], Maya Raad[1], Aimee C. McKinnon[1], Maria E. Moran-Diez[2], Peter C.H. Cheong[3], Michael Rostas[1]

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The genus *Beauveria* contains species well known as insect pathogens, and increasingly *B. bassiana* as plant endophytes. We have been using genomic and transcriptomic approaches to better understand

how *Beauveria* spp. interact with plants, controls sporulation and to identify the genes involved in the range of toxic metabolites produced. In our laboratory we have sequenced representatives of three species of *Beauveria, B. bassiana*, *B. caledonica* and *B. malawensis*. As expected, strains and species within *Beauveria* differ in their complement of genes encoding toxins. Comparative genomics across four species has also shown quite high genomic divergence for closely related species. Our research has included studies on the effect of *B. bassiana* endophytes on maize and *Arabidopsis*, which have shown strain specific and more general plant responses. Studies on the range of toxic metabolites in *B. bassiana* have shown that many compounds can be involved in insect effects. Transcriptomics of sporulating and non sporulating cultures has been used to study sporulation related events, as well as improving gene delimitation. For one species, we are nearing chromosome level assemblies, which should greatly assist future research.

Symposium Thursday 8.30 **121**

Cancelled

Symposium Thursday 9.00 **122-STU**

Genetic analysis of *Beauveria bassiana* JEF-007 as a biopesticide against bean bug

Se Jin Lee, Sihyeon Kim, Mi Rong Lee, Jong Cheol Kim, So Eun Park, Tae Young Shin, Baek Sehyeon, Jae Su Kim

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Entomopathogenic fungi are widely known as environment-friendly agents controlling agricultural insect pests. However, little consideration has been given to the study about the roles of fungal genes and genetic differences among the species in genome level. Herein, we have identified the genetic differences of entomopathogenic fungi using whole genome sequencing of *Beauveria bassiana* (Bb) and tried to understand the interaction between fungus and insect using RNA-seq. We have obtained the whole genome sequence of Bb JEF-007 using PacBio sequencing technology and compared the transcriptomes of Bb JEF-007 and bean bug, *Riptortus pedestris* before and after the fungal infection using Hiseq 2000 system. The whole genome of Bb JEF-007 was 36.5 Mb and the protein coding genes were 10,857. The Bb JEF-007 genome was compared with *B. bassiana* ARSEF2860. When highly identical genes between the two Bb isolates were subjected to real-time PCR, their transcription levels were different, particularly in heat shock protein 30 (hsp30) gene which is related to conidial thermotolerance. In several *B. bassiana* isolates, chitinases and trypsin-like protease genes involved in pathogenesis were highly conserved, but other genes showed noticeable sequence variation within the same species. In the RNA-seq analysis, 2,381 Bb JEF-007 genes were up-regulated and 2,303 Bb JEF-007 genes were down-regulated after the infection. In R. pedestris, 3,588 genes were up-regulated and 3,296 genes were down-regulated after the infection. In conclusion, the comparison of Bb JEF-007 and ARSEF2860 in whole genome level revealed that the expressed gene sequences in both strains were the same, but the expression levels could be different. In RNA-seq results, we have established a platform for the functional study of genes involved in the infection of the fungus by comparing gene expression levels in the insects and fungus before and after infection.

Symposium Thursday 9.30 **123**

Cancelled

Contributed papers Maui 1 & 2

Thursday 8.00-10.00

Viruses 4

Moderator**:** Abd-Alla A.M.M. and Chejanovsky Nor

Contributed paper Thursday 8.00 **124**

Mechanism of salivary gland hypertrophy virus (SGHV) infections: Prerequisite for tsetse and trypanosomosis control

Meki, I.K.[1, 2], Kariithi, H.M.[1, 3], Parker, A.G.[1], Vreysen M.J.B.[1], Ros, V.I. [2], Vlak, J.M.[2], van Oers, M.M.[2] and **Abd-Alla A.M.M**.[1]

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Tsetse flies control and the African trypanosomiasis via the sterile insect technique (SIT) requires mass production of the target tsetse species. This mass production can be severely hindered by infections with the *Glossina pallidipes* Salivary gland hypertrophy virus (GpSGHV; Hytrosaviridae). Although GpSGHV asymptomatically infects many Glossina species, it is in G. pallidipes that the symptomatic infections, which are characterized by the salivary gland hypertrophy (SGH) syndrome, are preferentially triggered. Our research efforts aim to identify the factors that trigger SGH outbreaks and how GpSGHV infection remains latent/asymptomatic within natural populations and colonized flies. We have determined how diverse GpSGHV strains are amongst tsetse species both under field and laboratory settings, and how GpSGHV is able to evade tsetse immune surveillance to induce SGH outbreaks in colonized

G. pallidipes. Firstly, we show that although GpSGHV infects at least seven *Glossina* species, the distribution, diversity and prevalence of the virus variants is highest in *G. pallidipes*. It therefore appears that GpSGHV has evolutionarily reached a stable but dynamic equilibrium with *Glossina* species other than *G. pallidipes*, which may account for SGH outbreaks in this tsetse species. These results imply that *G. pallidipes* is the major contributing species for evolution and interspecies spread of GpSGHV in tsetse mass rearing facilities, and therefore care must be taken in the handing of this tsetse species in facilities that rear multiple tsetse species. Secondly, we present the first evidence of the involvement of the host RNA interference (RNAi) in establishment of asymptomatic/latent GpSGHV infections. In *G. pallidipes* species, the efficacy and/or regulation of the RNAi machinery components appear to be impaired. Finally, we show that GpSGHV infections alter the miRNA profile in *G. pallidipes* that may potentially target the immune response genes thereby causing symptomatic infections. A few key factors/genes in *G. pallidipes* immune surveillance have been identified (e.g. the immune deficiency (IMD) pathway), which can be rationally exploited to suppress the threat of SGH outbreaks in *G. pallidipes* colonies and therefore a milestone in the implementation of SIT-mediated control of tsetse and trypanosomiasis in sub-Saharan Africa.

Contributed paper Thursday 8.15 **125-STU**

Deciphering the population structure of genotype mixtures of CpGV field isolates by next generation sequencing techniques and improved sequence analyses methods

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Along the antagonistic process of evolution between baculovirus and its insect host, virus-host interaction undergoes a continued mutual adaptation. This process results in a selection of virus populations, which differ geographically and even functionally. To explore this knowledge on molecular level, we focused on naturally occurring isolates of Cydia pomonella granulovirus (CpGV). CpGV infects larvae of codling moth (*Cydia pomonella* L.) and has been developed to one of the most successful commercial baculovirus biocontrol agents, being applied in the field all over the world. In recent years three types of field resistance as well as the existence of resistance-breaking CpGV isolates have been discovered, providing an ideal model for studying baculovirus-host adaptation. In the present study, 23 CpGV isolates, including geographic isolates from all over the world as well as commercial products and pure genotype bacmids, were sequenced using Illumina next generation sequencing techniques. On average, 91.97% of 2,208,599 QC-passed reads ($Q \geq 30$) of each isolate were mapped separately against CpGV-M reference genome using a workflow on the Galaxy server of Julius Kühn Institute. The obtained data set represents the complete known genetic diversity of CpGV. The genotype composition of all isolates was determined via single nucleotide polymorphism (SNP) analysis. Based on the position and frequency of the detected 762 SNPs, including 443 previously identified SNPs and 319 new SNP positions, a genetic fingerprint database was constructed and correlated with bioassay data which deciphered resistance-breaking signatures. Results from resistance tests confirmed that the genotype composition impacted on CpGV efficiency against resistant codling moth larvae. Therefore, the established methods did not only led to the characterization of new CpGV isolates but further allowed the unambiguous identification and quantification of genotype mixtures. In general, this study overcomes the limitation of currently used phylogenetic methods for isolate identification of baculoviruses, which are primarily based on a majority consensus sequence. It provides novel analysis tools to decipher the molecular complexity of genotype mixtures in virus isolates, thus depicting the population structure of naturally occurring baculoviruses isolates in a more adequate form.

Contributed paper Thursday 8.30 **126**

Construction of a reverse genetics system of *Dendrolimus punctatus* cypovirus and its application

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Dendrolimus punctatus cypovirus (DpCPV), belonging to the genus Cypovirus within the family Reoviridae, is an important pathogen of D. punctatus, which is considered as the most destructive pest of pine forest worldwide. DpCPV has genome consisting of 10 linear double-stranded RNA segments. In order to establish a reverse genetics system of DpCPV, we assigned cDNAs of the 10 genomic segments into 3 reverse genetics vectors where expression of each segment controlled by a T7 RNA polymerase promoter, terminated by an hepatitis delta ribozyme sequence and a T7 RNA polymerase terminator. Meanwhile, we constructed a AcMNPV Bacmid (AcBac-T7pol-RdRp-Δvp80) which expressed T7 RNA polymerase and DpCPV RNA-dependent RNA polymerase (RdRp) based on a vp80-knockout AcBacmid. Following co-transfection of Sf9 cells with the 3 vectors and AcBac-T7pol-RdRp-Δvp80, typical

occlusion bodies (OBs) with infectivity against D. punctatus larvae were successfully recovered. Additionally, infectious rDpCPV OBs expressing the eGFP was obtained when the egfp gene flanked with 5' and 3' UTR of the 10th genomic segment was inserted into a vector as the 3 reverse genetics vectors manner and mixed with the 3 vectors. Construction of reverse genetics system of DpCPV can be utilized to explore viral replication and pathogenesis, and facilitate the development of novel bioinsecticides and expressing system for exogenous genes.

Contributed paper Thursday 8.45 **127-STU**

Developmental resistance to natural infection by DCV in *Drosophila melanogaster*

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An increase in resistance to pathogenic viral infections during the larval development has been observed in numerous insect species, posing a major barrier for effective biological control strategies. A developmental resistance to natural oral infection was described in larvae of *Drosophila melanogaster* and its most lethal virus, the Drosophila C Virus (DCV, Dicistroviridae), shortly after DCV was first isolated; but no further research has been carried out so far in this area. Furthermore, the dynamics of the antiviral defences in larvae of holometabolous insects have been widely overlooked, and remain obscure. The *Drosophila*-DCV model is then an ideal starting point for elucidating the basic mechanistic changes directing the increase in resistance to viral infections. The peritrophic matrix (PM) acts as a physical barrier against ingested pathogens; however, its capacity to block the passage of viruses seems limited to late larval stages, at least in the baculovirus model where it has been studied. To evaluate its role in the *Drosophila*-DCV model, in this research we first established a new protocol for oral infection of *Drosophila* larvae, and tested the susceptibility of wild-type fly lines (Champetières, Oregon-RC, w^{1118} and y^1w^{67c23}) to DCV at 4 time points of development (recently hatched larvae, mid-L_1, mid-L_2 and early L_3). We found that a significant trend decrease ($p<0.001$) in the DCV-induced mortality occurs through time for all wild-type lines. We then used this infection protocol to evaluate the antiviral protection conferred by the PM at the same 4 time points, using mutant fly lines deficient for structural components of the PM (Transglutaminase$^{-/-}$ and Drosocrystallin$^{-/-}$), and compared their survival to their appropriate genetic controls. Finally, we induced the biochemical degradation of the PM by feeding larvae of the w^{1118} line with dextran sodium sulphate prior to oral DCV infection, and compared it to the PM mutants and the intact-PM control. These results provide a replicable protocol for oral infection of *Drosophila* larvae, and a better understanding of the antiviral role of the PM at different stages of the larval development.

Contributed paper Thursday 9.00 **128**

Identification of Osugroshi virus, a late male-killing virus in *Homona magnanima*

Ryosuke Fujita[1,2], Maki Inoue[3], Takumi Takamatsu[3], Hiroshi Arai[3], Mayu Nishino[3], Hironori Koyama[3], Nobuhiko Abe[3], Kentaro Itokawa[4], Madoka Nakai[3], and Yasuhisa Kunimi[3]
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A male-killing can be categorized into two groups: male-specific death in the embryonic stage (early male-killing) or in the larval or pupal stage (late male-killing). We have previously found late male-killing agents from *Homona magnanima* as two fragments of RNA, which were suspected to be a part of virus. We first established the laboratory strain of *H. magnanima* with late male-killing phenotype (late strain), possessing late male-killing RNA. We extracted RNA from adult females of late strain and carried out sequencing analysis. The results of the sequence analysis demonstrated that the late male-killing agents were new members of Partitiviridae, a double-stranded RNA virus. The identified viral sequences were composed of three segments or RNA1 encoding RNA-dependent RNA polymerase (RdRp), one segment of RNA2 encoding capsid protein, and one segment of RNA3 with unknown function. Based on the phylogenetic analysis of the RdRp sequences, we designated the viruses as Osugoroshi virus (OGV) 1–3. The sequences of OGVs RdRp were significantly different each other and not categorized in the previously defined genera of Partitiviridae, however, they showed similarity with recently identified insect-derived virus-like sequences. We then purified OGV virions from adult female of *H. magnanima* late strain by sucrose gradient centrifugation. The electron microscopy showed that the virions had round (possibly icosahedral) structure with the size about 30 nm in diameter, which was consistent with typical partitiviruses. The inoculation of purified OGV virions to female larvae of *H. magnanima* normal strain produced late male-killing phenotype in their progenies which were infected with OGVs. Our findings are the first report for identification of late male-killing virus and isolation of partitivirus from insects

Contributed paper Thursday 9.15 **129**

Replication of Apis Rhabdovirus-1\Bee Rhabdovirus-1, a negative-sense RNA virus of pollinators, in *Apis mellifer*a and *Varroa destructor*

Levin Sofia[1,2], Galbraith David[3], Sela Noa[4], Erez Tal1, Grozinger Christina M[3] and Chejanovsky Nor[1]
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Positive-sense single stranded RNA viruses are most of the viruses shown to be shared across bee species. However new emerging data from metagenomics studies reveals that negative-sense RNA viruses are present as well and may be shared among pollinators. In honey bees (*Apis mellifera*), many viruses are transmitted - and their impacts exacerbated - by the parasitic *Varroa destructor* mite. Studying the viral populations of *Apis mellifera* and *Bombus impatiens* from bee populations in Israel and the United States we found the negative-sense RNA enveloped virus, Apis rhabdovirus-1\Bee rhabdovirus-1 (ARV-1\BRV-1) in these two bee species, as well as in *V. destructor* mites. Via quantitative Real-time PCR we discovered that in individual honey bees and mites ARV-1\BRV-1 can reach high titers (10^7-10^8 viral genomic copies). Furthermore, screening of honey bee colonies across Israel showed that the prevalence of the virus was about 20 %. Determination of the presence of the complementary sense RNA-strand indicated that ARV-1\BRV-1 replicates in *A. mellifera* and *V. destructor*. Our results suggest that *Varroa* mites could act as an ARV-1 vector; however, this may not be an absolute requirement for transmission among co-foraging bee species since we found ARV-1\BRV-1 in *B. impatiens* as well. These results contribute to our knowledge on the diversity of viruses that can infect bee communities.

Contributed paper Thursday 9.30 **130**

Cryo-EM structure reveals cylindrical nucleocapsids from two Polydnaviruses

, Ji-Hui Cui[1,2,3,†], Ya-Bin Chen[1, 3,†], Ming Li[1,2,3,†], Qiu-Chen Cai[1,2,3], Li-Dan Zhang[1,2,3], Zi-Yun Lu[4], Jian-Cheng Li[4], Qi-Shun Zhu[1,3], Gang Ji[5,*], **Kai-Jun Luo**[1,2,3,]
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Bracovirus and Ichnovirus, the two polydnavirus genera, are groups of enveloped, double-stranded DNA insect viruses associated with parasitic wasps that develop within the body of lepidopteran larvae. Bracoviruses (BVs) have been previously described as enveloped particles possessing a specific cylindrical nucleocapsid of a consistent diameter but variable length that is composed of end structures and a tail-like structure. However, this description is based on results from conventional purification and electron microscopy (EM) staining methods, which considerably distort the native viral structure. Here, we used a cryo-EM analysis to reveal the near-native morphology of two nucleocapsid-carrying model Bracovirus species, Microplitis bicoloratus bracovirus (MbBV) and Microplitis mediator bracovirus (MmBV). The improved preservation revealed that MbBV and MmBV nucleocapsids have remarkable cap structures in two distal regions with relatively higher electron-density, previously called “end structures.” Based on our data, we have renamed them as “end-cap structures.” Adjacent to the end-cap structures are two electron-lucent rings, and, within the nucleocapsid, there is a cylindrical area of intermediate electron-density. Some nucleocapsids were uniformly electron-dense and had a distinctive “helix-tail-like structure,” which was not observed by prior negative-staining EM. Furthermore, cryo-EM revealed inconsistent nucleocapsid diameters of 34–69.9 nm in MbBV and 46–69.9 nm in MmBV, and the largest observed cylindrical area length was expanded to 126 nm. Based on our observations of BV nucleocapsids with near-native morphology, we propose an updated description of their typical appearance; they have variable diameters and lengths and contain end-cap structures, electron-lucent rings, and, sometimes, a helix-tail-like structure.

Contributed papers Maui 3

Thursday 8.00-10.00

Microbial Control 3

Moderator: Mary Babercheck

Contributed paper Thursday 8.00 **131-STU**

Laser Capture Microdissection to study iron homeostasis gene expression in *Bacillus cereu*s during *Galleria mellonella* midgut infection

Laurent Consentino[1], Agnès Réjasse[1], Nicolas Crapart[2,3], Christophe Buisson[1], Claudia Bevilacqua[2], Christina Nielsen-Leroux[1]
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Iron acquisition is essential for pathogenic bacteria in DNA synthesis, enzyme activities, and respiration. However, free iron is toxic for organisms: it is bound to other molecules like hemoproteins, transferrin or stored in ferritin. To overcome this lack of free iron, bacteria possess several systems to acquire bound iron, by surface proteins or secreted iron chelating siderophores. In the gut iron homeostasis is complex since host cells, commensal microbiota and food-iron are interacting to maintain this balance and little is known about genes involved during gut infection. Our work addresses this purpose in *Bacillus cereus*, (closely related to the insect pathogen *Bacillus thuringiensis*). The presentation will focus on genes related to iron homeostasis expressed during gut infection in the larvae of the insect model, *Galleria mellonella* (Greater wax moth). To perform these analyses, we adapted Laser Capture Microdissection to collect *B. cereus* cells in cryo-sections of previously force fed germ-free larvae and assessed targeted gene expression by RT-qPCR from the recovered mRNA. The results showed modulation of several iron homeostasis related genes at 16h (bacteria colonising the intestine surface) compared to 3h (bacteria in the lumen) post infection. Further analyzes are ongoing to characterize physiological and chemical markers in the gut environment.

Contributed paper Thursday 8.15 **132**

Plant functional traits, but not diversity, and soil characteristics affect the occurrence of *M. robertsii* in an organic cropping system

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Organic farmers rely largely on cultural practices, such as crop rotation and cover cropping, and on biological control to manage pests. Winter cover crops add diversity to agroecosystems and can benefit soil conservation and health, retention and supply of soil nutrients, regulation of arthropods, and crop yields. Within the framework of a three-year experiment to investigate ecosystem services and disservices associated with winter cover crops during the transition of an agronomic cropping system to organic management, we examined the effects of crop, cover crop species and diversity, arthropods, agronomic management and soil properties on the occurrence of *Metarhizium* in central Pennsylvania, USA. We used sentinel insect assays using *Galleria mellonella* L. to determine the relative occurrence of *Metarhizium* in soil samples from treatment plots. *M. robertsii* was the only species detected, and therefore, cover crop diversity was not related to the species diversity. Cover crop treatment did not affect detection of *M. robertsii*, but detection was lower in monocultures and mixtures containing brassica cover crops compared to those with legume cover crops. *M. robertsii* was detected more frequently in corn than in soybean, and in standing cover crop (pre-termination) compared to post-cover crop termination samples in the corn phase of the rotation. Using multiple regression analysis, we found that cover crop biomass in the previous fall, the biomass of weeds in the current season (spring), percent silt, electrical conductivity, and activity-density of ground beetles were positively associated with and together explained 28.32% of the variation of percentage mortality sentinel *G. mellonella* by *M. robertsii* in pre-termination samples. In post-termination samples, soil labile C and electrical conductivity were positively associated, and activity-density of mites and percent sand were negatively associated with *M. robertsii* and together explained 21.92% of the variation in detection of *M. robertsii*. The complex interactions of multiple biotic and abiotic factors that shape the soil community require further research to develop an understanding of how to manage production systems and practices to promote and conserve biological control in the soil.

Contributed paper Thursday 8.30 **133**

Transmission of *Beauveria bassiana* and *Metarhizium anisopliae* to male *Bactrocera tryoni* via para-pheromone lures and subsequent transmission to females

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Queensland fruit fly (*Bactrocera tryoni*) is a major pest of fruit and fruiting vegetables in Australia. It is a significant market access and trade barrier for many exporting commodities. The pest is generally managed by insecticide cover-sprays and postharvest treatments. However, the withdrawal of some pesticides has restricted control options. Para-pheromone lures, also known as male lures combined with toxicants are effective at attracting and killing male *B. tryoni* flies (male annihilation), but they do not kill the female flies. Male annihilation must be combined with other treatments to kill females, such as protein baiting. In caged laboratory experiments, we examined novel ways to lure and kill male *B. tryoni* with male lures impregnated with *Beauveria bassiana* or *Metarhizium anisopliae*. The lures were manufactured in a similar way to "non-woven fungal bands"; dental cotton rolls were submerged into a liquid fungal culture, which was then allowed to sporulate. The cotton rolls were air-dried and injected with the male lure (Cue lure). Sexually mature adult virgin male *B. tryoni* were placed into cages (30/cage) containing the fungal lures or negative controls (lures without fungus). After 24hrs, the lure was removed and virgin female *B.*

tryoni flies (30/cage) were placed into the cages with the males to determine if the fungi could be passed on from male to female via direct contact. In these experiments, the fungi impregnated lures were highly successful at transmitting *B. bassiana* to male flies, with up to 100% mortality (94% confirmed infection by sporulation on cadavers) from one *B. bassiana* isolate. However, the subsequent transmission of fungi from male to female flies was not as high, with a maximum of only 20% of confirmed infected females for the same *B. bassiana* isolate. The rates of *M. anisopliae* transmission were lower than the *B. bassiana* lures.

Contributed paper Thursday 8.45 **134**

Efficacy of entomopathogenic fungi and *Bacillus thuringiensis* isolates against the invasive Fall Armyworm, *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae)

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Maize is a major staple food for over 300 million people in sub-Saharan Africa (SSA). Sustainable productivity of this primary crop is constantly threatened by various abiotic and biotic constraints that inflict 15 – 80% loss in Africa. This loss has been further aggravated recently with the invasion by Fall Armyworm, *Spodoptera frugiperda* (FAW). Due to lack of adequate management strategies, immediate responses by growers and governments are based on rampant use of pesticides which in most cases is ineffective and unsustainable in the long term. In the pursuit to develop potent biopesticides, efficacy of some selected entomopathogenic fungi and *Bacillus thuringiensis* (Bt) isolates were evaluated on egg and larval stages of FAW. Nineteen Bt strains were screened against second instar larvae of FAW through maize leave treatment at a single discriminating concentration of $1x10^8$ cfu ml^{-1} using Potter spray tower, of which seven were found highly effective causing 100% mortality 7 days post-treatment, and with lethal time (LT_{50}) values ranging between 2.33 - 6.50 days. In addition, the pathogenicity of 21 fungal isolates from three different genera (*Metarhizium, Beauveria* and *Isaria*) were assessed against second instar FAW, but only *B. bassiana* isolate ICIPE 676 caused moderate mortality of 30 %. However, when tested against the eggs of FAW at $1x10^8$ conidia ml^{-1}, isolates of *M. anisopliae*, ICIPE 78, ICIPE 40 and ICIPE 20 outperformed all the others by reducing the eggs hatchability by 87, 83 and 79.5%, respectively. In addition to the eggs mortality, the fungal isolates also induced mortality to the newly emerged larvae from the fungus treated eggs 7 days post-emergence. *Metarhizium anisopliae* isolates, ICIPE 41 and ICIPE 7 outperformed all the others by causing 96.49 and 93.66% larval mortality respectively. ICIPE 78 which has been already commercialized as Achieve® against spider mites by Real IPM in partnership with icipe, could therefore be used as a potential ovicidal biopesticide to suppress FAW population in Africa.

Contributed paper Thursday 9.00 **135**

Susceptibility of *Spoladea recurvalis* (Lepidoptera: Crambidae) to entomopathogenic fungal and *Bacillus thuringiesis* (Bt)- based biopesticides

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Spoladea recurvalis (Lepidoptera: Crambidae) is a major pest of amaranths. The larvae voraciously feed on amaranth leaves resulting to 100% yield loss if not controlled. Chemical control is the main management strategy for *S. recurvalis* but with detrimental effects to consumers, the environment and non-target organisms. Development of insecticide resistance is also a major concern. Microbial control using entomopathogenic fungi (EPF) and *Bacillus thuringiensis* (Bt)-based biopesticides are considered as sustainable alternatives to synthetic chemical insecticides. The aim of this study was to evaluate the effects of various entomopathogenic fungal isolates and commercial based *Bacillus thuringiensis* subsp. *kurstaki* product Halt® on this key amaranth pest. Twenty-four EPF isolates from three genera (14 *Metarhizium anisopliae*, 9 *Beauveria bassiana* and 1 *Isaria fumosorosea*) were screened in the laboratory to assess their pathogenicity against second instar larvae of *S. recurvalis*. Only *M. anisopliae* ICIPE 30 reached a moderate threshold, causing 58.3 % larval mortality. In the Bt-based biopesticide product Halt® bioassay, <50 % mortality was recorded on *S. recurvalis* larvae. Combined application of *M. anisopliae* ICIPE 30 and Bt did not cause any significant increase in larval mortality compared to separate applications of both products. However, all 11 isolates (8 *M. anisopliae*, 2 *B. bassiana* and 1 *I. fumosorosea*) tested against adult *S. recurvalis* were pathogenic to the pest, with *M. anisopliae* ICIPE 30 and *B. bassiana* ICIPE 725 causing the highest mortality of 92 and 83 % respectively. Results of this study suggest that M. anisopliae ICIPE 30 was the most potent candidate for the management of adult *S. recurvalis* and could be integrated in *S. recurvalis* – IPM technology. The present study is the first laboratory based report on the susceptibility of *S. recurvalis* to entomopathogenic fungi and Bt and their potential for amaranth lepidopteran management.

Contributed paper Thursday 9.15 **136**

Impact of geographic location on mosquito microbiota

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The microbial communities inhabiting the mosquito body play an import role in host biology and are considered potential tools for mosquito control. However, the forces that shape these microbial communities are poorly understood. To gain a better understanding of how host geography influences the composition and diversity of mosquito microbiota, we performed a survey of whole-body microbial communities in mosquito samples collected from six U.S. states using HiSeq sequencing of the 16S rRNA gene. The microbial composition and diversity was heavily influenced by the site of mosquito collection, suggesting that host geography plays an important role in modulating the mosquito microbiota. Geographic variations in microbial composition and diversity in mosquitoes may have important implications on vector competence and transmission dynamics of mosquito-borne pathogens.

Contributed paper Thursday 9.30 **137**

A plant-derived protein with insecticidal activity against Western Corn Rootworm

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Damage caused by western corn rootworm (WCR; *Diabrotica virgifera virgifera*) can result in high yield loss in corn production regions of North America. Corn rootworm traits developed based on the toxins expressed by *Bacillus thuringiensis* have been an important tool utilized by growers to maximize productivity. However, the potential for development of resistance in the field has generated the urgency to develop new traits that can complement or replace existing products. In this regard, a protein, IPD079Aa, was isolated from the fern *Huperzia phlegmaria* demonstrating activity against WCR. Homology based screening led to the identification of a related protein (IPD079Ea) with WCR activity from the fern *Ophioglossum pendulum*. Interestingly, IPD079Ea showed a broader spectrum of activity (Southern Corn Rootworm; *Diabrotica undecimpunctata*) than IPD079Aa and excellent root protection when expressed in transgenic corn. For these reasons, more detailed studies were initiated to understand the mode of action of these proteins. Specifically, binding studies were conducted using brush border membrane vesicles (BBMVs) from WCR which demonstrated that IPD079 proteins bind to BBMVs with moderate affinity through the same receptor, but do not share binding sites with proteins that are surrogates for WCR commercial traits (Cry3 or Cry34/35) or with the novel *Pseudomonas* WCR-selective IPD072Aa. A similar binding analysis was conducted with IPD079Ea using BBMVs from lepidopteran insects showing no binding under any condition tested. WCR gut fluid stability testing demonstrated that IPD079Ea is proteolytically stable after 2h of treatment, but was rapidly degraded in gut fluid from lepidopteran insects. Overall these results show that IPD079Ea has great potential for development into a trait with a new mode of action for protection against root damage caused by WCR.

Contributed paper Thursday 9.45 **138**

Cancelled

Cross Divisional Symposium (Virus & Diseases of Beneficial Insects) Pipeline

Thursday 13.30-15.30

White Spot Syndrome Virus – Emergence and control

Organisers/Moderators: Kelly Bateman & Just Vlak

Symposium Thursday 13.30 **139-STU**

Overview of WSSV and its emergence

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White spot syndrome virus (WSSV) is a member of the Nimaviridae family and Whispovirus genus. The size of virion is (343.3±85.2) nm × (135.9±29.0) nm, of that the nucleocapsid is (352.2±80.4) nm × (79.2±27.4) nm. The envelope is consist of a monolayer of base membrane and a fuzz layer. The nucleocapsid of WSSV has 15 closed lateral circles. The total number of capsomers in a capsid is 616. Buoyant densities of virions and nucleocapsids of WSSV were measured as (1.233±0.017) g/cm^3 and (1.306±0.025) g/cm^3, respectively. The MW of

WSSV measured by structural calculation is (2.65±1.17)×10^6 KD, of which (56.7±23.7)% is the envelope and the tail, (43.3±16.5)% is nucleocapsid (including DNA), and (7.6±2.4)% is the genome. The sedimentation coefficient of WSSV in water is (3970±1197) S. WSSV has an extremely broad host range, infecting all decapod crustaceans (shrimp, crayfish, lobsters, and crabs), regardless of habitat and life stage. The infection of WSSV can be transmitted vertically and horizontally. WSSV causes systemic infection in cuticle epithelium, hematopoietic tissue, gills, connective tissue, antennal gland, and early hemocytes, but do not infect in epithelium of hepatopancreatic tubules and midgut, myocytes, later hemocytes, oocytes, spermatocytes, and lymphoid organs, etc. The infection of WSSV cause calcium carbonate deposit on inner surface of cuticles, loosen cuticles from epithelium, tachycardia, accelerated breathing, and granulated crystalline columns. The early study showed increasing pathogenicity of WSSV to crayfish *Procambarus clarkii* during continuous passages of generation 1 to generation 6. Higher virulent isolates of WSSV had a LD50 to crayfish *P. clarkii* ranging from 2.29×10^3 copies/g (BW) to 6.22×10^3 copies/g (BW) and a LT50 to *Litopenaeus vannamei* ranging from 3.5 d to 4.8 d, while lower virulent isolates of WSSV had a LD50 ranging from 1.50×10^5 copies/g (BW) to 2.02×10^6 copies/g (BW) and a LT50 ranging from 5.2 d to 5.4 d. Mutations in the genome of WSSV including deletions in ORF23/24, ORF1415, and transposase, repeat unit numbers and single nucleotide polymorphisms (SNPs) in ORF75, ORF94, ORF125, and SNPs, single nucleotide mutations and single nucleotide insertions in structural and functional genes. Viral attachment proteins and its cellular receptors and other molecules involved in interaction between WSSV and host cells were reported.

Symposium Thursday 14.00 **140**

White spot disease outbreak in farmed prawns in Queensland, Australia in 2016

Peter Mohr[1], Nicholas Moody[1], Mark Crane[1], Debbie Eagles[1] Stephen Wesche[2], Kerrod Beattie[2], Allison Crook[2]

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On 22 November, 2016 Biosecurity Queensland were notified of a minor mortality event in a pond on a prawn farm at Alberton in southeast Queensland. A week later, mortality had reached 90% with adjacent ponds also suffering significant losses. On 30 November, the Biological Sciences Laboratory in Brisbane detected white spot syndrome virus (WSSV) in samples from mortalities in the first pond and the detection was confirmed the following day by the CSIRO Australian Animal Health Laboratory in Geelong. On 1 December, a meeting of the Aquatic Consultative Committee for Emergency Animal Diseases was convened to consider the proposed Queensland response plan, and Emergency Powers of Inspectors under the Queensland Biosecurity Act 2014 were activated. On 7 December, WSSV was confirmed in wild-caught prawns from the lower reaches of the Logan River. Despite eradication efforts on affected farms, by mid-February 2017 the disease had spread to all the other prawn farms in the region. In March 2017 and again in March 2018, WSSV has also been confirmed in prawns and crabs in Moreton Bay. During the first six months of the outbreak response greater than 50,000 samples from farmed prawns and wild-caught crustaceans were tested for the presence of WSSV. Surveillance activities in the affected area as well as the rest of the country are currently being undertaken to determine the extent of the virus's spread.

Symposium Thursday 14.30 **141**

Wild type relative of the most important viral pathogen in global aquaculture

K.S. Bateman[1,2], J. Bojko[2], J. Vlak[3], R. Kerr[1,2], K.F. Clark[4,5], S.E. Stewart-Clark[5], P. Byrne[6], S.J. Greenwood[4], D. Bass[1], G.D. Stentiford[1,2], R. van Aerle[1,2].

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White Spot Syndrome Virus (WSSV) remains the most significant viral disease impacting the sustainability and growth of the global penaeid shrimp farming industry. WSSV is a large dsDNA virus, although most commonly associated with penaeid shrimp farmed in warm waters, the virus is also able to infect, cause disease and kill a wide range of other decapod crustaceans from temperate regions (including lobsters, crabs, crayfish and shrimp). The origin of the virus in wild host reservoirs has been speculated but detection of familial relatives has not occurred to date, with WSSV residing as sole member of the family Nimaviridae. Viruses which are morphologically similar to WSSV have been identified in portunid crabs in Europe and it has been suggested that these may be ancestral forms of WSSV. Here, we

report detection of an extant wild-type relative of WSSV infecting wild crabs from temperate waters. The virus causes similar clinical signs to WSSV infection in shrimp and phylogenetic analyses demonstrates that this new virus is the closest known relative of WSSV and places the virus as a second member of the Nimaviridae family. The discovery and description (pathology, morphology, genome) of a novel virus infecting crabs not only allows for expansion of this single-taxon family but further, shines a light on the potential origin of WSSV in wild animal reservoirs. It is quite likely that further members of the Nimaviridae family exist in wild populations of other decapod crustaceans, these will continue to be discovered as sequencing methods and sampling programmes are developed.

Symposium Thursday 15.00 **142**

Potential future therapies for WSSV

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White spot syndrome virus (WSSV) has been a long standing threat to global shrimp aquaculture. Despite the urgent industry need to control the virus, practical prevention and therapeutic strategies in shrimp are unavailable. Mechanistic insights into WSSV-host interactions have led to the development of a variety of molecular-based preventative and therapeutic approaches to mitigate shrimp mortality caused by WSSV. This presentation summarizes recent advances in therapies for WSSV and discuss prime bottlenecks for farm-scale applications. One currently studied method of high interest for protecting shrimp against viral infection relies on the post-transcriptional gene silencing mechanism called RNA interference (RNAi) that is mediated by complementary base pairing between gene-specific constructs of double stranded RNA (dsRNA) and target transcripts. The second approach takes advantage of a diverse array of antimicrobial peptides that shrimp naturally produce as the first line of defense upon viral infection. These small 15-100 amino acids cationic amphipathic peptides have been shown *in vitro* to exhibit antimicrobial activity and directly kill pathogens. The third strategy builds on our current understanding on how WSSV is taken up by shrimp cells. In order to enter a host cell, structural proteins on WSSV envelope must be recognized by host receptors. One of such interactions of current interest is between WSSV VP28 envelope protein and *Penaeus monodon* endosomal protein Rab7 (PmRab7). At the laboratory scale, intramuscular injection of either VP28 or PmRab7 significantly increased survival of shrimp upon WSSV challenge. Nonetheless, none of the aforementioned methods have been implemented at the farm level due to the lack of cost-effective production pipelines for the active molecules and simple and cost-effective delivery methods. Many research groups are trying to address the latter challenge by developing nanocontainers that can encapsulate those functional ingredients and can be mixed into normal shrimp feed.

Contributed papers Maui 3

Thursday 13.30-15.30

Nematodes 1

Moderators: Patricia Stock and Ivan Hiltpold

Contributed paper Thursday 13.30 **143**

Effect of temperature on the infectivity of different entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) isolated from natural ecosystems

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Pathogenicity of entomopathogenic nematodes (EPNs) against the greater wax moth (*Galleria mellonella*) larvae was evaluated at six different temperatures ranging from 10°C to 35°C, under laboratory conditions. Entomopathogenic nematodes used in the bioassay belong to four species *Steinernema feltiae, S. ichnusae, Heterorabditis bacteriophora* and *Oscheius onirici*, were naturally isolated from Mediterranean countries (Southern Italy and Lebanon). Infective Juvenile (IJs) were put in contact with *G. mellonella* larvae in Petri dishes and mortality rates were recorded after 72 hours. The purpose of the study was to evaluate the temperature range in which the EPNs caused larval mortality. Highest mortalities were recorded at 15°C and 20°C. All species failed at lower temperatures except for *Steinernema ichnusae* ItS-SAR4, which caused 7% mortality. At 35°C *S. ichnusae* maintained its infectious activity

(24%) along with *Heterorhabditis bacteriophora* ItH-LU1 (38%); both were isolated from Italy and were more efficient at high temperatures than the remaining Lebanese isolates.

Contributed paper Thursday 13.45 **144**

Natural occurrence of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in areas infested by *Popillia japonica* (Coleoptera, Scarabaeidae) in Northern Italy

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The Japanese beetle *Popillia japonica* Newman was found, for the first time in continental Europe, in Northern Italy in 2014 in the Ticino Valley between Piedmont and Lombardy regions. This scarab beetle feeds on more than 300 species of plants thus being considered a severe pest for agriculture and landscape. The necessity to limit the spreading of this alien insect is crucial to avoid a biological invasion in all the Italian territory and European countries. A field survey was carried out in spring 2017 in the infested areas with an eco-friendly approach in order to spot the natural occurrence of entomopathogenic nematodes (EPNs) potentially able to control the Japanese beetle spreading.
A total of 155 soil samples, each homogenized from 5 subsamples, were collected from an equivalent number of localities in perennial meadows and forest areas lying in the Ticino Valley.
Out of the 36 EPN wild species/strains recovered by the use of *Galleria mellonella* baiting technique, 3 strains belonged to the genus *Heterorhabditis* and 33 to *Steinernema.* Laboratory screening tests were conducted to evaluate the ability of EPNs to control *P. japonica*. At first, the pathogenicity of all the nematode strains was assessed against larvae of *Galleria mellonella* in Petri dish single-larva assays with a concentration of 300 infective juveniles (IJs) per larva. Subsequently, the seven most performing *Steinernema* and the three *Heterorhabditis* strains were tested against *P. japonica* third instar larvae in semi-field experiments. As an outcome, we observed that the *Heterorhabditis* strains/species caused a mortality ranging from 40% to 84%, whilst the *Steinernema* ones caused at most 52% mortality. In conclusion, this study confirmed once again that EPNs may be valuable biological control agents against *P. japonica* to be tested within integrated pest management field experiments. It was also interesting to note how these territories along the Ticino river are particularly rich in these organisms, thus demonstrating a good natural ability to withstand these white grubs.

Contributed paper Thursday 14.00 **145**

Cancelled

Contributed paper Thursday 14.15 **146**

Photo-biology: a tool to identify nematodes

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Identification of nematodes can be especially challenging and requires advanced skills. Preparing slides for morphological identification involves several steps and the use of harmful chemicals in addition to human dexterity, and good microscopy facilities. The recent development of several specific primers makes molecular identification and quantification very accurate. However, the extraction of DNA and its molecular amplification can still be problematic for some laboratories, both financially and technically. Here, we explored a new avenue in nematode identification. Based on the assumption that photo-biological signatures are species specific, we have exposed *Heterorhabditis bacteriophora, Steinernema feltia*, and *S. carpocapsae* to various light wavelength (visible, UV and deep-UV spectrums) and recorded their spectral signatures. Using a hand-held spectrometer, we measured an optimal fluorescence of the three species using a 375 nm UV laser. This technique allowed a quick and repeatable differentiation between the three tested species. In addition, the use of deep-UV (200-300 nm) permitted an accurate evaluation of the nematode concentration of the samples. This approach still requires refinements but it could allow a very quick, low-cost and labour, and accurate identification and quantification of entomopathogenic and/or other nematode species.

Contributed paper Thursday 14.30 **147**

Symbiont-mediated thermal tolerance in *Steinernema puntauvense* (Honduras strain) nematodes

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The mutualism between *Steinernema* nematodes and their *Xenorhabdus* symbionts is linked to the fitness of both partners. Third-stage infective juvenile nematodes must withstand long-term

environmental stressors in the soil while searching for a new insect host. These abiotic stressors vary seasonally and regionally. In this study, we considered an experimental evolution approach to test the hypothesis that thermal tolerance in *Steinernema* nematodes can be mediated by their bacterial symbionts. For this purpose, we considered a tropical climate nematode-bacterium pair represented by *Steinernema puntauvense* (Honduras strain) and its bacterial symbiont *Xenorhabdus bovienii*. Three temperature conditions, 15, 25 and 35°C were considered for the symbiont temperature selection regime. Symbiont selection assays showed the bacteria grew more readily at and adapted more quickly 15°C than at 25 or 35 °C. Subsequently, *X. bovienii* adapted at 15°C were re-associated with the nematodes, and their virulence and reproductive fitness were assessed. Our results showed that symbionts selected for a colder temperature (15°C) conferred an increased fitness to the nematodes reared at this temperature and at a warmer temperature (25°C) when compared to nematodes reared with the unselected bacterial strain. Specifically, nematodes associated with the cold-selected strain had an increased virulence at 15 °C. Similarly, time to emergence of progeny was shorten. Progeny production of nematodes associated with the cold-selected *X. bovienii* strain also increased at 15 °C and 25 °C. These data demonstrate a proof-of-concept for the use of experimental evolution to select symbiotic bacteria to improve virulence in *Steinernema* nematodes. It also represents the first empirical evidence to support the symbiont-mediated environmental tolerance in this system. Additionally, the implications of influencing nematode fitness by manipulating their bacterial symbionts provides a new avenue to improve the formulation of entomopathogenic nematodes for biocontrol.

Contributed paper Thursday 14.45 **148**

Mermithid parasitism of shoot borer (*Conogethes punctiferalis*) infesting ginger and turmeric and its biocontrol potential

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A mermithid nematode parasitic to larvae of *Conogethes punctiferalis*, a serious pest of ginger and turmeric was recorded under field conditions during 2015 at Peruvannamuzhi (Kerala, India). The infection reached epizootic levels during July to September 2015 exceeding 50% mortality in host insect populations and the parasitism ranged from 18.2% to 80.6 % and 17.9% to 66.7% in *C. punctiferalis* collected from ginger and turmeric, respectively. The level of host parasitism by the mermithid was positively correlated with rainfall and negatively influenced by increase in temperature. Molecular analysis of the partial 18S SSU gene region and phylogenetic analysis with other mermithid sequences available in the GenBank indicated that the nematode was distantly related to *Hexamermis,* and to *Ovomermis sinensis* and Mermithid species reported to infect spiders, bumble bees and slugs. The low bootstrap support in the phylogenetic tree indicates that this nematode could be a species hitherto unreported. The epizootics caused by this mermithid nematode in the natural populations of *C. punctiferalis* suggest its potential as a biocontrol agent against a polyphagous pest of international importance. The results of our studies provide a basis for using this nematode as a biocontrol agent for developing IPM strategies against *C. punctiferalis*.

Contributed paper Thursday 15.00 **149**

Management of *Halyomorpha halys* by entomopathogenic nematodes in Georgia

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Halyomorpha halys, also known as the brown marmorated stink bug (BMSB), or simply the stink bug. Brown marmorated stink bug is a serious agricultural pest that has been readily causing damage to crops across the west and eastern Georgia. They feed on a wide array of plants including apples, apricots, Asian pears, cherries, corn, grapes, beans, peaches, peppers, tomatoes, and soybeans. This makes them extremely versatile as they do not require a specific plant to feed on. The adults are approximately 1.7 centimetres. They are various shades of brown on both the top and undersides, with gray, off-white, black, copper, and bluish markings. Various laboratory bioassays were conducted to determine the effectivity of entomopathogenic nematodes to control *Halyomorpha halys* (Stink bug). Adult *H.halys* (Stink bug) were screened for susceptibility to two introduced from Israel nematode species. *Halyomorpha halys* was found to be most susceptible to *Steinernema carpocapsae* and *Steinernema feltiae*, causing 34, 48, 84% and 22, 31, 66% mortality, respectively. Further bioassays illustrated a linear relationship between stink bug, mortality and the concentration of nematodes applied, with the highest level of control using a concentration of 500, 1000, 1500 infective juveniles (IJs)/insect. *Steinernema carpocapsae* proved able to locate and infect stink bug, quicker, than *Steinernema feltiae*. For all nematode species, the highest virulence was observed 34, 48, 84%, and 22, 31, 66% on the temperature 25°C and 1500 IJs/ml cm^2 concentration for *S. carpocapsae,* and *S. feltiae,* respectively. In conclusion, It was determined that *Halyomorpha halys* (Stink bug) can be controlled by *S. carpocapsae*, but further studies should be conducted at private houses, field and greenhouse conditions.

Contributed papers Maui 1 & 2
Thursday 13.30-15.30
Bacteria 3
Moderator: Marianne P. Carey

Contributed paper Thursday 13.30 **150**

Aedes cadherin is an essential gene targeted by Cry11A

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In *Drosophila*, E-cadherin, a constituent of sub-apical region (adherens junctions) plays an essential role in preservation of epithelial integrity. An orthologous cadherin is not present in *Aedes aegypti* mosquitoes, but distantly related cadherin-like proteins exist in *Aedes*. One of these, the *Aedes* cadherin (AeCad), has been characterized as a receptor for *Bacillus thuringiensis* subsp. *israelensis* (Bti) Cry11A toxins. However, its role in *Aedes* development and in Cry11A toxicity is not fully understood. In this study, we manipulated the cadherin gene using ZFN and TALEN. Even though gene deletions and heterozygotes were obtained, we could never obtain homozygous mosquito lines. Because ZFN and TALEN have much less off-target effects, we think the aecad gene is likely essential for *Aedes* development. In contrast in lepidopteran insects a similar cadherin appears to be non-essential since homozygous mutants are viable. To analyse its expression patterns in vivo we examined AeCad localization by gene tagging. We successfully EGFP tagged this protein using CRISPR-mediated homologous recombination. Confocal images showed high AeCad expression in the larval caeca and posterior midgut, where Cry11A binds, and low expression in the anterior gut where the Cry11A protein does not bind. The EGFP-tagged cadherin co-localizes with the detection of cadherin by AeCad-specific polyclonal antibodies, suggesting it is indeed the *Aedes* cadherin receptor that was tagged with EGFP. The EGFP-tagged cadherin proteins are only localized on the apical side of epithelium cells, distinct from that of snake skin, a membrane protein associated with smooth septate junctions. However, *Aedes* cadherin co-localizes with proteins that are expressed in the sub-apical region protein. The data suggests AeCad is not a cell junction protein but could be expressed in sub-apical regions as is the E-cadherin in *Drosophila.* Nevertheless the *Aedes* cadherin is an essential gene for mosquito development. A discussion of its potential role in mediating midgut cell response to Cry11A intoxication will also be presented.

Contributed paper Thursday 13.45 **151**

Role of *Bacillus thuringiensis* Cry1A toxins domains in the binding to the ABCC2 receptor from *Spodoptera exigua*

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Cry proteins from *Bacillus thuringiensis* (Bt) have been used to control insect pests either as formulated sprays or as in Bt-crops. However, field-evolved resistance to Bt proteins is threatening the long-term use of Bt products. The SeABCC2 locus has been genetically linked to resistance to a Bt bioinsecticide (XentariTM) in *Spodoptera exigua* (a mutation producing a truncated form of the transporter lacking an ATP binding domain was found in the resistant insects). Here, we investigated the role of SeABCC2 in the mode of action of Cry1Aa, Cry1Ab, Cry1Ac, Cry1Ca, two Cry1A-1Ca hybrids, and a Cry1Ac mutant by expressing the receptor in Sf21 cell line. Cell toxicity assays showed that Sf21 cells expressing SeABCC2 become susceptible to Cry1A proteins. In contrast, the Cry1Ac mutant had no effect on cell viability. The results with the Cry1A-1Ca hybrids suggest that domain II from Cry1Ab/c is crucial for the toxicity to Sf21 cells. Binding assays showed that the Cry1Ac binding is of high affinity and specific to cells expressing the SeABCC2 transporter. Heterologous competition experiments support a model in which domain II of Cry1Ab/c has a common binding site in the SeABCC2 protein, whereas domain III of Cry1Aa/b binds to a different binding site in the SeABCC2 protein.

Contributed paper Thursday 14.00 **152**

Scale-free genetic interaction networks in *Heliothis virescens* challenged with *Bacillus thuringiensis* toxin Cry1Ac

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Large scale genomic experiments allow analysis of regulatory pathways used by biological systems to transmit signals and coordinate multiple processes. These networking mechanisms allow the systems to respond and adapt to ever changing environments. It has been observed that transcription networks exhibit an approximately scale-free distribution, signifying the potential of transcription factors to regulate a multitude of target genes. These signaling networks are poorly understood in many organisms including the tobacco budworm (TBW, *Heliothis virescens*), which is a model insect for

studying insecticide resistance. The aim of the current research is to quantitatively describe a network of hundreds or thousands of interacting genes that will give us clues about the dynamic response of this pest to insecticidal proteins from *Bacillus thuringiensis* (Bt) toxins. Early fourth instar larvae of TBW were exposed to a sub-lethal dose of Cry1Ac and Illumina short reads were obtained from three pools of midguts harvested at 0, 120, and 480 min after exposure. Approximately 20 million reads from each replicate were mapped to 18,728 TBW genes and weighted co-expression networks that exhibit a scale-free topology were identified. The highly interactive modules obtained from this analysis identified some of the genes associated with Bt mode of action/resistance being co-regulated with those that may be directly or indirectly involved in the response to Bt toxins in TBW.

Contributed paper Thursday 14.15 **153**

Transcription of the cellobiose transport pathway is controlled by Sigma 54 and regulated by CelR in *Bacillus thuringiensis*

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Bacillus thuringiensis is a pathogen of Lepidoptera and Coleoptera pests. Cellobiose might be rich present in the midgut of Lepidoptera larvae which is feed on green leaves. Cellobiose-specific phosphotransferase system for cellobiose transport and utilization, which is encoded by cel gene cluster in *B. thuringiensis* (Bt) has been investigated. cel gene cluster consists of six genes, celA (encodes the cellobiose-specific EIIB component of PTS), celB (encodes cellobiose-specific EIIC component of PTS), celC and celE (encodes the cellobiose-specific EIIA component of PTS), celD (encodes β-6-P-glucosidase), and celR (encodes Sigma 54-dependent transcriptional activator). In this study, we analyzed the transcription and regulation of the cel gene cluster in Bt. RT-PCR analysis revealed that the celABCDE forms one transcriptional unit. The typical -12/-24 consensus sequence was located 55 bp from the transcriptional start site (TSS) of celA. β-galactosidase assay of promoter-lacZ fusion showed that the transcription of the celABCDE operon is induced by cellobiose and controlled by Sigma 54, and positively regulated by CelR. The transcription of cel operon is repressed by glucose via CcpA, and CcpA specifically bound to sequences within the celA promoter fragment. In the celABCDE and celR mutations, PTS activity was decreased and cellobiose utilization was abolished, suggesting that the cel gene cluster is essential for cellobiose transport and utilization. Cellobiose is potential carbon source as the component of cellulose widely existing in the environment. Clarifying the transcriptional regulation mechanism of cellobiose utilization pathway is contributing to studying the adaptation of Bt in environment, and especially in herbivorous insects.

Contributed paper Thursday 14.30 **154**

Polycalin is involved in the action mode of Cry2Aa toxin and resistance mechanism of Cry1Ac toxin in *Helicoverpa armigera* (Hübner)

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Receptor proteins on the brush border membrane of the insect midgut epithelium are involved in the mode of action of insecticidal Cry proteins from *Bacillus thuringiensis* (Bt), and the mutations of receptor proteins lead to resistance to Cry proteins. Polycalin has been identified as a binding protein of the Bt Cry1Ac toxin in several Lepidoptera including *Helicoverpa armigera*, but its role in the action mechanism of Cry2Aa and resistance mechanism to Cry1Ac are still unclear. In this study, we investigated the binding characteristics of polycalin from the midgut of H. armigera with Cry2Aa and its role involved in the toxicity of Cry2Aa. The results demonstrated that heterologously expressed *H. armigera* polycalin peptide could bind with Cry2Aa with high affinity *H. armigera* polycalin (Kd =32 nmol L-1). And the toxicity of Cry2Aa decreased by 27% after *H. armigera* larvae ingested polycalin antisera. Moreover, *H. armigera* polycalin peptide could also specifically bind with Cry1Ac (Kd =119 nmol L-1), and the expression of polycalin protein increased in Cry1Ac-resistant *H. armigera* than that in Cry1Ac-susceptible strain. These results suggested that polycalin could be a potential functional receptor for Cry2Aa, it plays an important role in the susceptibility of *H. armigera* to Cry2Aa, and polycalin might also be involved in resistance mechanisms to Cry1Ac in *H. armigera*.

Contributed paper Thursday 14.45 **155**

Cancelled

Contributed paper Thursday 15.00 **156**

Genome-wide analysis of ATP-binding cassette (ABC) transporters in the cotton bollworm, *Helicoverpa armigera*

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Insects are frequently exposed to toxic compounds including secondary metabolites produced by host

plants or pesticides artificially manufactured by man. Many insects have adapted to these toxins through the evolution of detoxification mechanism. However, the knowledge about the ATP-binding cassette (ABC) transporters, a multigene family involved in detoxification processes, is limited. To get a better understanding of this gene family, we measured the temporal and spatial expression of this gene family and the gene expression changes of the larvae fed on different hosts and different pesticides. The expression patterns of ABC genes showed developmental specific and tissues specific. The larval fed on corn, chili, cotton, soybean showed a different patterns of ABC gene expression. Similarly to different host plants, the larval fed on lambda-cyhalothrin, Abamectin, indoxacarb, chlorpyrifos, tebufenozide also had a strong influence on the ABC transporter gene expression. The highest number of differentially expressed genes was recorded in the treatment of lambda-cyhalothrin. HaOG200302 was only significantly up-regulated in the tebufenozide treatment. HaOG200310, HaOG200353 and HaOG200354 were common highly expressed in the treatment of Abamectin, indoxacarb and lambda-cyhalothrin. In this study, we analyzed the temporal and spatial expression profiles of the ABC genes. Moreover, the expression data of ABC genes fed on different hosts and different pesticides lay a foundation for future analysis of their function in detoxification process.

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Hiltpold	Lanilà	Accompanying Partner	USA

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The Society for Invertebrate Pathology acknowledges the support of: