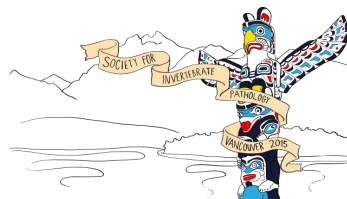


International Congress on Invertebrate Pathology and Microbial Control and the 48th Annual Meeting of the Society for Invertebrate Pathology

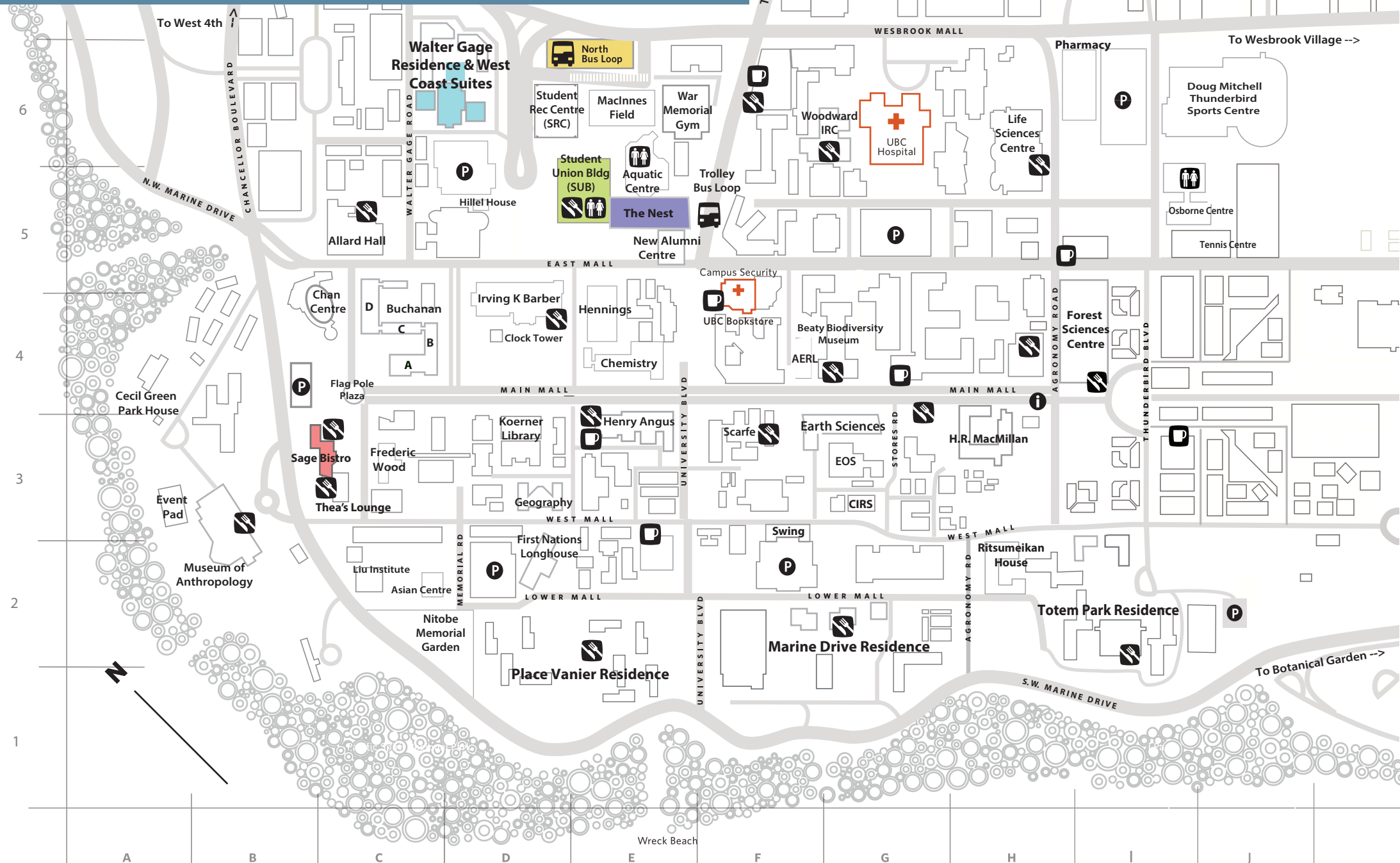


**Vancouver, Canada
August 9-13, 2015**



Society for Invertebrate Pathology 48th Annual Meeting August 9-13, 2015

University of British Columbia
Vancouver BC



- Accommodation**
- C6 Walter Gage Residence and West Coast Suites
- Meeting Space**
- E5 The Nest
- Breakfast**
- E5 Pacific Spirit Cafeteria In The Student Union Building
- Mixer**
- B3 Sage Bistro

- Bus Loop
- Parking
- Public Washrooms
- G6 **UBC Hospital**
- F4/5 **UBC CAMPUS SECURITY**

SIP 2015 Vancouver

SATURDAY – August 8		
14:00 – 20:00	Registration	Gage Reception
SUNDAY – August 9		
7:30 – 18:00	Registration	Gage Reception
8:30 – 17:30	OECD-CRP Symposium	2301
	<i>'Microsporidia in the animal to human food chain: An international symposium to address chronic epizootic disease'</i>	
9:00 – 17:00	SIP Council meeting	2504
10:30 – 11:00	BREAK	
12:20 – 13:30	LUNCH	
13:00 – 17:00	Workshop: Bacteria Division	2306/9
	<i>'Regulatory considerations for the commercialization of new insecticidal proteins'</i>	
	<ul style="list-style-type: none"> • Current insights on Bt insecticidal protein specificity and future directions – <i>J. Jurat-Fuentes, N. Crickmore</i> • Proteins 101: structure, function, and evolution – <i>J. Jez</i> • Protein sequences, structures and functions – rules for divergence and rules for conservation – <i>Adam Godzik</i> • Modelling of insecticidal toxins and their potential interactions: Challenges and aspirations – <i>C. Berry, N. Crickmore</i> • Safety considerations derived from Cry34/35Ab1 structure and function – <i>K. Narva, N. Storer, R. Herman</i> 	
14:40 – 15:00	BREAK	
	<ul style="list-style-type: none"> • Case study of a novel wCRW insecticidal protein from <i>Chromobacterium</i> sp. – <i>K. Sampson</i> • Biochemical characterization of parasporin-4 and effects of the pro-parasporin-4 diet on the health of mice – <i>S. Okumura, H. Kogo, K. Inouye, E. Mizuki</i> • Considerations for the safety assessment of novel insect control proteins: A regulatory perspective – <i>P. MacDonald</i> • New insecticidal proteins: Optimization and specificity – <i>W. Moar, J. Haas, A. Evdokimov, J. Baum, A. Silvanovich, Y. Yin, D. Bowen, K. Glenn, A. Evan</i> 	
16:20 – 17:00	DISCUSSION	
18:00 – 21:00	Mixer	Sage Bistro
MONDAY – August 10		
7:30 – 18:00	Registration	Outside Great Hall
8:00 – 10:00	Opening Ceremony	Great Hall
	Todd Kabaluk & Mark Goettel – Organizing Committee	
	Peter Krell – President, SIP	
	Welcome Addresses; Award Presentations	
	Founders' Lecture James Becnel – Chair of Founders' Lecture Committee Honoree: Phyllis T. Johnson Lecturer: G. Stentiford	
10:00 – 10:30	BREAK	
10:30 – 12:30	Plenary Symposium	Great Hall
	<i>'Insect pathogens in nature: Ecology and evolution'</i>	

all meetings are in The Nest

Meeting at a Glance

	<ul style="list-style-type: none"> • How sea stars get wasted: Evidence of a viral etiology and host response to sea star wasting disease – <i>C. Burge</i> • Symbiont-mediated defense against parasitic nematodes in <i>Drosophila</i> – <i>S. Perlman</i> • No nematode is an island: Interactions between entomopathogenic nematodes and other organisms – <i>C. Griffen</i> • The ecology of virulence in insect associated bacteria: Field experiments and experimental evolution – <i>B. Raymond</i>
12:30 – 14:00	LUNCH (on your own)
12:30 – 14:00	J. Invertebr. Pathol. Board lunch meeting 2508
14:00 – 16:00	Symposium: Microsporidia/Diseases of Beneficial Invertebrates 2301
	<i>'Microsporidia as emerging pathogens'</i>
	<ul style="list-style-type: none"> • The complex relationship between microsporidia and fungi – <i>P. Keeling</i> • Fish microsporidians: Emerging pathogens or emerging knowledge? – <i>M. Freeman</i> • Understanding phylogenetic relationships among relationships among species in the <i>Nosema/Vairimorpha</i> clade: What does genetic similarity say about host switching in the microsporidia? – <i>W-F. Huang</i> • Emergent pathogens of invertebrates: Environmental sampling to identify novel parasite lineages – <i>B. Williams</i> • Investigations into the composition of the microsporidian polar tube – <i>L. Weiss</i>
14:00 – 16:00	Contributed Papers
	Bacteria 1 2306/9
	Microbial Control 1 2311
16:00 – 16:30	BREAK
16:30 – 18:30	Symposium: Fungi/Microbial Control 2301
	<i>'The (underestimated) value of applied research: Moving the theoretical to the practical'</i>
	<ul style="list-style-type: none"> • Why biopesticides sometimes fail – <i>R. Gwynn, M. Brownbridge, T. Glare</i> • Multiple roles, so what should we measure? An ecological approach to promote the contribution of fungal entomopathogens in pest management within the agro ecosystem – <i>N. Meyling</i> • Research and development of biological crop protection products – <i>R. royalty, D. Manker</i> • Adapting field trials for microorganisms – in practice – <i>E. Ladurner</i> • How do we improve efficacy monitoring or biopesticides? – <i>T. Glare, M. Brownbridge, R. Gwynn</i>
16:30 – 18:30	Contributed Papers
	Viruses 1 2306/9
	Microsporidia 2311
20:00 – 22:00	Division meetings
	Microbial Control 2301
	Diseases of Beneficial Invertebrates 2306/9
	+ Workshop: <i>Environmental DNA</i>
TUESDAY – August 11	
8:00 – 10:00	Symposium: Bacteria 2301
	<i>'Mechanisms of field resistance to Bt pesticides and Bt-crops'</i>
	<ul style="list-style-type: none"> • Bt resistance in <i>Plutella</i> – too many trees? – <i>N. Crickmore</i> • Multiple resistance mechanisms selected in cabbage looper populations resistant to Dipel – <i>P. Wang</i> • Pink bollworm resistance to Bt cotton: Similar mechanisms in the lab and the field – <i>J. Fabrick</i>

	<ul style="list-style-type: none"> • Mechanism of <i>Spodoptera frugiperda</i> resistance to Cry1Fa in <i>Bt</i> corn – <i>J. Jurat-Fuentes</i> • Characterization of potential resistance mechanisms to Cry3Bb1 in western corn rootworm (<i>Diabrotica virgifera virgifera</i>) – <i>J. Haas</i> 	
8:00 – 10:00	Contributed Papers	
	Diseases of Beneficial Invertebrates 1	2311
	Viruses 2	2306/9
10:00 – 10:30	BREAK	
10:30 – 11:30	Contributed Papers	
	Bacteria 2	2301
	Diseases of Beneficial Invertebrates 2	2311
11:45	Excursion+BBQ buses leave	Gage Residence
15:30	BBQ only buses leave	Gage Residence
18:00	5k race	Cheakamus Center
19:00	BBQ	Cheakamus Center

WEDNESDAY – August 12

8:00 – 10:00	Symposium: Viruses	2301
	<i>'Advances in host and insect virus genomics'</i>	
	<ul style="list-style-type: none"> • Macro- and Micro-evolutionary trends in baculoviruses – <i>J. Jehle</i> • Alphabaculoviruses: Host transcriptome responses to infection – <i>G. Blissard</i> • Polydnviruses: From discovery to current insights – <i>M. Strand</i> • Discrovirus – hijacking the host translational machinery – <i>E. Jan</i> • Insect metagenomics-based discovery of novel, small RNA viral genomes – <i>S. Liu</i> 	
8:00 – 9:45	Contributed Papers	
	Microbial Control 2	2306/9
	Nematode 1	2311

BREAK

10:00 – 10:30	BREAK	
10:30 – 12:30	Symposium: Nematodes/Bacteria	2306/9
	<i>'Intracellular response to bacteria and bacterial toxins'</i>	
	<ul style="list-style-type: none"> • Insecticidal action, cellular interactions and response of combinations of <i>Photorhabdus</i>-insect related (Pir) and <i>Bacillus thuringiensis</i> Crystal (Cry) toxins – <i>A. Castagnola</i> • Response to Cry1Ac intoxication in midgut cells of <i>Heliothis virescens</i> larvae – <i>C. Oppert</i> • Syringe-like infection mechanism of bacterial ABC toxins revealed in molecular detail – <i>C. Gatsogiannis</i> • <i>Caenorhabditis elegans</i> nck-1 plays a distinct and specific role in defense against bacterial pore-forming toxins – <i>A. Sitaram</i> • The cell biology of <i>Wolbachia</i> - filarial nematode interactions and the dark side of symbiosis – <i>W. Sullivan</i> • <i>Photorhabdus</i>: light without heat – <i>N. Waterfield</i> 	

10:30 – 12:30	Contributed Papers	
	Fungi 1	2311
	Viruses 3	2301

LUNCH (on your own)

12:30 – 14:00	Student Workshop w/lunch	2311
	<i>'Writing scientific articles: what reviewers and publishers are looking for'</i>	

14:00 – 16:00	Symposium: Fungi	2301
	<i>'Endophytic entomopathogenic fungi: "pro-biotic" microbial associates of plants?'</i>	
	<ul style="list-style-type: none"> • Endophytic entomopathogenic fungi as "plant probiotics": An important tool in protecting and promoting plant health – <i>C. Keyser</i> • Entomopathogenic fungi as endophytes: Interactions with plants and herbivores – <i>S. Vidal</i> • Trading insect nitrogen for photosynthate: Carbon translocation from a plant to an insect pathogenic, endophytic fungus – <i>M. Bidochka</i> 	

	<ul style="list-style-type: none"> • <i>Metarhizium</i> as a multifactorial plant growth promoter – <i>R. St. Leger</i> 	
14:00 – 15:00	Nematodes Workshop	2311
14:00 – 16:00	Contributed Papers	
	Viruses 4	2306/9

BREAK

16:00 – 16:30	BREAK	
16:30 – 18:30	POSTER SESSION	Great Hall
20:00 – 22:00	Division meetings:	
	Fungi	2311
	Viruses + Workshop: <i>Definition of virus species concept</i>	2301
	Microsporidia + Workshop: <i>War and Peace: the comparative impact of morphology- and sequence-based phylogenies on practical taxonomy and evolutionary history of microsporidia</i>	2314
	Bacteria	2306
	Nematodes	2309

THURSDAY – August 13

8:00 – 10:00	Symposium: Nematodes	2301
	<i>'Recent advances in entomopathogenic nematode infection behavior: inside and outside'</i>	
	<ul style="list-style-type: none"> • Advances in entomopathogenic nematode dispersal and host-finding behavior – <i>D. Shapiro-Ilan</i> • Sex, age and following the leader drive infection dynamics of entomopathogenic nematodes – <i>E. Lewis</i> • Impact of infection behavior on lethal male fighting in <i>Steinernema</i> – <i>C. Griffin</i> • The stability of virulence in insect parasitic nematodes is determined by social interactions – <i>B. Raymond</i> 	

8:00 – 10:00	Contributed Papers	
	Fungi 2	2306/9
	Microbial Control 3	2311

BREAK

10:00 – 10:30	BREAK	
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10:30 – 12:30	SIP Business Meeting	Great Hall
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LUNCH (on your own)

12:30 – 14:00	Symposium: Microbial Control	2301
	<i>'Synergies enabling the registration & adoption of biological pest controls – the role of governments, academic programmes, and industry'</i>	
	<ul style="list-style-type: none"> • Facilitating the registration and adoption of biological pest controls in Canada – <i>T. Laengle</i> • The IR-4 biopesticide and organic support program – <i>B. Barney</i> • How does academia contribute to registration and adoption of biological control agents – a European perspective – <i>J. Eilenberg</i> • Perils and pitfalls of product development and commercialization: An industry perspective – <i>R. Martin</i> • Panel Discussion 	

14:00 – 16:00	Contributed Papers	
	Viruses 5	2306/9
	Bacteria 3	2311
16:15 -	Student Affairs Business Meeting	2311
18:00 – 1:00	Banquet	Great Hall

Table of Contents

(sections are hyperlinked / click bottom of pages to return here)

UBC Campus Map	3
Meeting at a Glance	4
SIP Officers & SIP Divisions	8
SIP Committees	9
SIP 2015 Organizing Committee	
<hr/>	
2015 PROGRAM	11
Sunday	13
Monday	14
Tuesday	17
Wednesday	19
POSTERS	22
Thursday	30
<hr/>	
2015 ABSTRACTS	35
Monday	36
Tuesday	47
Wednesday	55
POSTERS	70
Thursday	102
<hr/>	
Author Index	113
Acknowledgment	125
Sponsors of SIP	Back cover

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President

Peter Krell

University of Guelph, Department of Molecular and Cellular Biology
50 Stone Rd East, Guelph, ON N1G 2W1, CANADA
Phone: 519 824 4120 x53368 Email: pkrell@uoguelph.ca

Vice President

Johannes Jehle

Federal Research Center for Cultivated Plants, Julius Kuehn Institute, Institute for Biological Control, Heinrichstr. 243, Darmstadt, 64287, GERMANY,
Phone: +49 (6151) 407-220 Email: johannes.jehle@jki.bund.de

Past President

Jørgen Eilenberg

Department of Plant and Environmental Sciences, University of Copenhagen,
Thorvaldsensvej 40, Frederiksberg C, DK 1871, DENMARK
Phone: +45 35 33 2692 Email: jei@plen.ku.dk

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Department of Entomology, Penn State University
University Park, PA 16802, USA
Phone: 814-863-2982 Email: meb34@psu.edu

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UC Cooperative Extension, University of California
2156 Sierra Way, Ste., San Luis Obispo, CA 66502, USA
Phone: 805-788-2321 Email: skdara@ucdavis.edu

Ed Lewis

Department of Entomology, University of California Davis
One Hutchison Ave, Davis, CA 95616, USA
Phone: 530-754-7659 Email: eelewis@ucdavis.edu

Albrecht Koppenhofer

Entomology Dept., Blake Hall, 93 Lipman Drive
New Brunswick, NJ 08901-8524, USA
Phone: 848-932-9802 Email: koppenhofer@aesop.rutgers.edu

Monique van Oers

Wageningen University, Lab. of Virology
Droevendaalsesteeg, Wageningen, 6708 PB, THE NETHERLANDS
Phone: 31-317-485082 Email: monique.vanoers@wur.nl

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P.O. Box 11, Marceline, MO 64658, USA
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Fungi: Chad Keyser & Joanna Fisher
Microbial Control: Patricia Golo
Microsporidia: Thomas Steele
Nematodes: John G. McMullen
Virus Division: Yasumasa Saito &
Gianpiero Gueli Alletti

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Joan Cossentine (Registration Coordinator)
Deborah Henderson (Social Activities Co-ordinator)
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Patricia Stock
Monique van Oers
Surendra Dara
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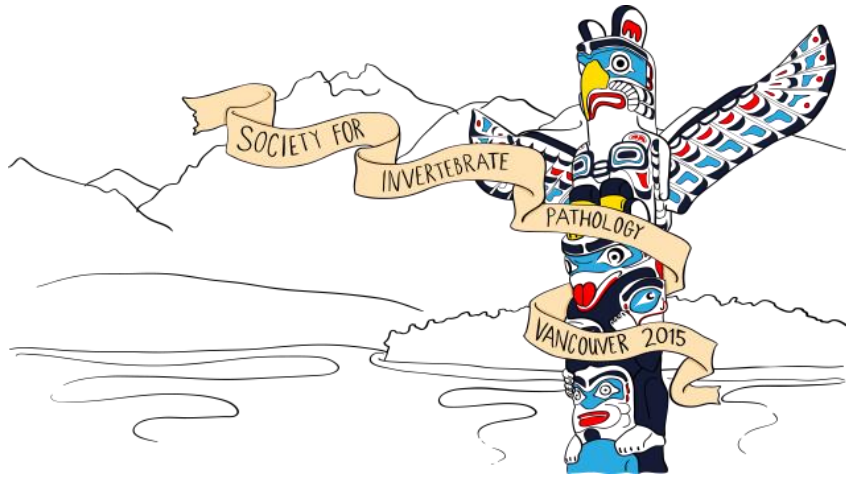
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2015 PROGRAM



-
- *Attendants shall **not take pictures** from projections during the presentations*
 - *The abstracts included in this book should not be cited in print without the author's permission*

STU Indicates **STUDENT** presentation

000 Indicates number of **ORAL** presentation

BA - 00 Indicates abstract number for **POSTER** presentation

SATURDAY – August 8

14:00 – 20:00 Registration Gage Residence Reception

SUNDAY – August 9

7:30 – 18:00 Registration Gage Residence Reception

9:00 – 17:00 SIP Council Meeting 2504

18:00 – 21:00 Mixer Sage Bistro

OECD-CRP Satellite Symposium Sunday 8:30 – 17:30
2301

Microsporidia in the Animal to Human Food Chain: An International Symposium to Address Chronic Epizootic Disease

Organizers: James Becnel, Leellen Solter, Grant Stentiford, Louis Weiss

Sponsored by the Organisation for Economic Co-operation and
Development-Co-operative Research Programme (OECD-CRP) and
the Society for Invertebrate Pathology

Session 1: Microsporidia – a general Introduction

Chair: *Grant Stentiford*

8:30 Introduction – *Grant Stentiford*

8:40 Introduction to the OECD/CRP. Stressors in the global
food chain and the importance of pathogens

– *Primal Silva*, Animal Health Science Directorate, Canada
and Member Scientific Advisory Body, OECD.

8:50 Parasites in food chains – *Kristina Rösel*, International
Livestock Research Institute, CGIAR Consortium.

9:10 Introduction to Microsporidia – *James Becnel*, USDA-ARS,
Gainesville, FL, USA.

9:30 The Microsporidia: Where did they come from and
where are they going – *Patrick Keeling*, Canadian
Institute for Advanced Research.

Session 2: Microsporidiosis in humans

Chair: *Louis Weiss*

9:50 Microsporidiosis in humans – an emerging issue?
– *Louis Weiss*, Albert Einstein College of Medicine, NY.

10:10 Is global immunosuppression linked to rising burdens of
microsporidiosis in human and animal populations?
– *Elizabeth Didier*, Tulane University.

10:30 – 11:00

BREAK

11:00 How do Microsporidia exploit the biochemistry and
physiology of the host cell? – *Bryony Williams*,
University of Exeter.

Session 3: Microsporidiosis in terrestrial animals

Chair: *James Becnel*

11:20 Microsporidiosis in farmed animals and terrestrial
wildlife – their roles in zoonoses – *Louis Weiss*,
Albert Einstein College of Medicine, NY.

11:40 Microsporidiosis in companion animals – their role in
zoonoses – *Karen Snowden*, Texas A & M.

12:00 Microsporidia as regulators of insect populations and
disease agents in mass-reared insects – a future threat
to edible insect cultivation? – *Susan Björnson*, St. Mary's
University, Halifax, Canada.

12:20 – 13:30

LUNCH

Session 4: Microsporidiosis in aquatic animals

Chair: *Grant Stentiford*

13:30 Microsporidiosis in wild fish – an emerging issue?
– *Michael Kent*, Oregon State University.

13:50 Wild and cultured fish as potential sources of zoonotic
infections in humans – *Mark A. Freeman*, University of
Malaya.

14:10 Pathogens of aquatic arthropods – focus on the
Enterocytozoonidae – *Grant D. Stentiford*, European
Union Reference Laboratory for Crustacean Diseases,
Cefas, UK.

14:30 Clues for multiple-taxa lifecycles from invertebrate
research – *Yuliya Sokolova*, Louisiana State University.

14:50 – 15:20

BREAK

Session 5: Microsporidian role in pollinator health

Chair: *Leellen Solter*

15:20 Is microsporidian infection/disease becoming more
common in bumble bees? – *Mark Brown*, Royal Holloway,
University of London.

15:50 Interactions of Microsporidia with the global honey bee
population – *Leellen Solter*, Illinois Natural History Survey,
University of Illinois.

Session 6: Future look and final discussion

Symposium Organizers

16:00 Current and future models for microsporidian research
– *Emily Troemel*, University of California, San Diego.

16:30 Discussion panel

Workshop Sunday 13:00 – 17:00
Bacteria 2306/9

Regulatory Considerations for the Commercialization of New Insecticidal Proteins

Moderators: William Moar and Ken Narva

13:00 Current insights on *Bt* insecticidal protein specificity and
future directions – *Juan Luis Jurat-Fuentes¹*, *Neil
Crickmore²*, ¹Department of Entomology and Plant
Pathology, University of Tennessee, Knoxville, TN, USA;
²School of Life Sciences, University of Sussex, Brighton,
UK.

13:20 Proteins 101: Structure, function, and evolution – *J. Jez*,
Washington University, Department of Biology, St. Louis,
MO 63130, USA.

13:40 Protein sequences, structures and functions – rules for
divergence and rules for conservation – *Adam Godzik*,
Bioinformatics and Structural Biology Program, Sanford-
Burnham Medical Research Institute, La Jolla, CA, USA.

14:00 Modelling of insecticidal toxins and their potential
interactions: Challenges and aspirations – *Colin Berry¹*,
Neil Crickmore², ¹Cardiff School of BioSciences, Cardiff
University, Cardiff, CF10 3AT, UK; ²School of Life Sciences,
University of Sussex, Brighton, UK.

14:20 Safety considerations derived from Cry34/35Ab1
structure and function – *Kenneth E. Narva*, *Nick Storer*,
Rod Herman, Dow AgroSciences, LLC, Indianapolis,
IN, USA.

14:40 – 15:00

BREAK

- 15:00 **Case study of a novel wCRW insecticidal protein from *Chromobacterium* sp.** – *Kimberly Sampson*, Bayer Crop-Science, Raleigh-Durham, North Carolina, USA.
- 15:20 **Biochemical characterization of parasporin-4 and effects of the pro-parasporin-4 diet on the health of mice** – *Shiro Okumura¹, Hironori Koga², Kuniyo Inouye³, Eiichi Mizuki¹*, ¹Biotechnology and Food Research Institute, Fukuoka Industrial Technology Center, Fukuoka, Japan; ²Division of Gastroenterology, Department of Medicine, Kurume University School of Medicine, Fukuoka, Japan; ³Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Kyoto, Japan; ⁴Inouye Laboratory of Enzyme Chemistry (iLEC), Kyoto, Japan.
- 15:40 **Considerations for the safety assessment of novel insect control proteins: A regulatory perspective** – *Phil MacDonald*, Plant and Biotechnology Risk Assessment Unit, CFIA, Ottawa, Ontario, Canada.
- 16:00 **New insecticidal proteins: Optimization and specificity** – *William J. Moar, Jeff Haas, Artem Evdokimov, Jim Baum, Andre Silvanovich, Yong Yin, Dave Bowen, Kevin Glenn, Adam Evan*, Monsanto Company, St. Louis, Missouri, USA.
- 16:20 – 17:00 DISCUSSION

18:00 – 21:00 Mixer Sage Bistro

MONDAY – August 10

7:30 – 18:00 Registration Outside Great Hall

Great Hall Monday 8:00 – 10:00

Opening Ceremony and SIP Founders' Lecture

8:00 **Opening Ceremonies**
Welcoming addresses
 Todd Kabaluk and Mark Goettel, Meetings Co-Chairs
 Peter Krell, President, SIP

Award presentations
 Andreas Linde, Chair, Awards and Student Contest Committee

Founders' Memorial Lecture
 James Becnel – Chair, Founders' Lecture Committee
 Honoree: Phyllis T. Johnson
 Lecturer: Grant Stentiford

10:00 – 10:30 BREAK

Plenary Symposium Monday 10:30 – 12:30
 Great Hall

Insect Pathogens in Nature: Ecology and Evolution

Moderators: Jenny Cory and Alida Janmaat

- 10:30 **1 How sea stars get wasted: Evidence of a viral etiology and host response to sea star wasting disease** – *Colleen A. Burge*, Team SSWD, Team EIMD, The Institute of Marine and Environmental Technology, University of Maryland Baltimore County, Baltimore, MD, USA.
- 11:00 **2 Symbiont-mediated defense against parasitic nematodes in *Drosophila*** – *Steve J. Perlman*, Department of Biology, University of Victoria, BC, Canada.
- 11:30 **3 No nematode is an island: Interactions between entomopathogenic nematodes and other organisms** – *Christine T. Griffin*, Biology Department, Maynooth University, Maynooth, County Kildare, Ireland.
- 12:00 **4 The ecology of virulence in insect associated bacteria: Field experiments and experimental evolution** – *Ben Raymond*, Imperial College London, Silwood Park Campus, Ascot, Berks, SLS 7PY, UK.

12:30 – 14:00 LUNCH (on your own)

12:30 – 14:00 JIP Editorial Board Meeting 2508

Symposium Monday 14:00 – 16:00
 Microsporidia/Diseases of Beneficial Insects 2301

Microsporidia as Emerging Pathogens

Moderators: Kelly Bateman and Susan Bjørnson

- 14:00 **5 The complex relationship between microsporidia and fungi** – *Patrick J. Keeling*, Department of Botany, University of British Columbia, Vancouver, BC, Canada V6T 1Z4.
- 14:20 **6 Fish microsporidians: Emerging pathogens or emerging knowledge?** – *Mark A. Freeman*, Institute for Experimental Pathology at Keldur, University of Iceland, Reykjavik, Iceland.
- 14:40 **7 Understanding phylogenetic relationships among species in the *Nosema/Vairimorpha* clade: What does genetic similarity say about host switching in the microsporidia?** – *Wei-Fone Huang¹, James Becnel², Leellen Solter¹*, ¹Illinois Natural History Survey, Prairie Research Institute at the University of Illinois at Urbana-Champaign, IL, USA; ²United States Department of Agriculture, Agricultural Research Service, Gainesville, FL, USA.
- 15:10 **8 Emergent pathogens of invertebrates: Environmental sampling to identify novel parasite lineages** – *Bryony A. P. Williams¹, Kristina Hamilton¹, David Bass²*, ¹Biosciences, College of Life and Environmental Sciences, University of Exeter, Exeter, UK; ²Centre for Environment, Fisheries & Aquaculture Science, Weymouth, Dorset, UK.
- 15:30 **9 Investigations into the composition of the microsporidian polar tube** – *Louis M. Weiss, Kaya Ghosh, Bing Han*, Departments of Medicine and Pathology, Albert Einstein College of Medicine, Bronx, NY, 10461, USA.

Contributed Papers Monday 14:00 – 16:00
 2306/9

Bacteria 1

Moderators: Ken Narva and David Heckel

- 14:00 **10 Resistance to *Bacillus thuringiensis* Cry2Ab toxin in *Helicoverpa* spp. is conferred by mutations in a novel ABC transporter** – Wee Tek Tay¹, Rod J. Mahan¹, Thomas K. Walsh¹, Sharon Downes², William J. James¹, Siu Fai Lee³, Annette Reineke⁴, Adam K. Williams³, Karl J. H. Gordon¹, David G. Heckel⁵, ¹CSIRO, Black Mountain Laboratories, Canberra, ACT, Australia; ²CSIRO, Australian Cotton Research Institute, Narrabri, NSW, Australia; ³Department of Genetics, University of Melbourne, Parkville, VIC, Australia; ⁴Institute for Phytomedicine, Geisenheim University, Geisenheim, Germany; ⁵Department of Entomology, Max Planck Institute for Chemical Ecology, Jena, Germany.
- 14:15 **11-STU Functional range of insect ABCC transporters as a Cry toxin receptor** – Haruka Endo^{1,2}, Shiho Tanaka¹, Ryoichi Sato¹, ¹Graduate School of Bio-Applications and Systems Engineering, Tokyo University of Agriculture and Technology, Koganei, Tokyo, Japan; ²Research Fellow of Japan Society for the Promotion of Science.
- 14:30 **12-STU Cry1A toxins cause rapid cell lysis in a clonal stable non-lytic expression system, expressing ABC-C2 and cadherin** – Anne Karpinski, Yannick Pauchet, David Heckel, Department of Entomology, Max Planck Institute for Chemical Ecology, Jena, Germany.
- 14:45 **13 Expressing a lepidopteran ABCC2 gene in transgenic *Drosophila* causes Bt Cry1Ac susceptibility without requiring a cadherin-like protein receptor** – Tristan Stevens, Simon W. Baxter, School of Biological Sciences, University of Adelaide, Australia.
- 15:00 **14 Differential toxicity of Cry1Ca and Cry1Ac to *Spodoptera exigua* (Lepidoptera: Noctuidae)** – Youngjin Park¹, Salvador Herrera², Yonggyun Kim¹, ¹Department of Bioresource Sciences, Andong National University, Andong 760-749, Korea; ²Department of Genetics, Universitat de València, Dr Moliner 50, 46100 Burjassot, Spain.
- 15:15 **15-STU Models for the interaction between cadherin-like receptor BT-R1 and three Cry1A family toxins from *Bacillus thuringiensis*** – Diogo Martins-de-Sa¹, Fernando C. A. Fonseca², Wagner A. Lucena², Patricia B. Pelegrini², Maria Fatima Grossi-de-Sa², ¹Cellular Biology, Department, University of Brasília, Brasília, Brazil; ²Embrapa Genetic Resources and Biotechnology, Brasília, Brazil.
- 15:30 **16 A high-throughput, in vitro assay for *Bacillus thuringiensis* Cry proteins** – Michi Izumi Wilcoxon, Jaclyn Dennis, Sabina Lau, Weiping Xie, You You, Song Leng, Ryan Fong, Takashi Yamamoto, Plant Protection, Ag Biotechnology, DuPont Pioneer, Hayward, CA, USA.
- 15:45 **17 Transcriptome response of *Aedes aegypti* against *Bacillus thuringiensis* and the pathway for enhancing the mosquitocidal activity** – Wu Songqing^{1,2}, Liu Zhaoxia², Wang Yafang², Xu Zhuoying², Guo Yajie², Xiong Yueting², Mou Yan², Zhu Xiaoli², Zhang Feiping¹, Zou Shuangquan¹, Zhang Lingling², Guan Xiong², ¹Collage of Forestry, Fujian Agriculture and Forestry University, Fuzhou, 350002, People's Republic of China; ²Key Laboratory of Biopesticide and Chemical Biology, Ministry of Education, Fujian Agriculture and Forestry University, Fuzhou, 350002, People's Republic of China.
- 14:00 **18 Autodissemination strategy for field suppression of *Bactrocera dorsalis* (Diptera: Tephritidae) using *Metarhizium anisopliae*-based biopesticide on mango** – Sunday Ekesi, Nguya K. Maniania, International Centre of Insect Physiology and Ecology (icipe), Nairobi, Kenya.
- 14:15 **19 Development of granules based on the biomass of liquid fermented *Metarhizium anisopliae*, *Isaria fumosorosea* or *Beauveria bassiana* for control of soil dwelling pests** – Dietrich Stephan¹, Medea Buranjadze², Tanja Bernhardt¹, Juliana Pelz¹, Johannes Schäfer¹, Christopher Seib¹, ¹JKI-Institute for Biological Control, Heinrichstrasse 243, 64287 Darmstadt, Germany; ²Agricultural University of Georgia, 240, D. Agmashenebeli Alley, Tbilisi 0159, Georgia, USA.
- 14:30 **20-STU Can pheromone enhance the transmission of *Metarhizium brunneum* in *Agriotes obscurus* click beetles?** – Joyce P. S. Leung¹, Jenny S. Cory¹, J. Todd Kabaluk², Alida F. Janmaat³, ¹Department of Biological Sciences, Simon Fraser University, Burnaby, BC, Canada; ²Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, Agassiz, BC Canada.
- 14:45 **21 Less effort - more efficient: An attract and kill strategy for wireworm control in potato** – Mario Schumann¹, Anant Patel², Stefan Vidal¹, ¹Georg-August-University Goettingen, Department of Crop Sciences, Goettingen, Germany; ²University of Applied Sciences, Department of Engineering and Mathematics, Bielefeld, Germany.
- 15:00 **22 *Drosophila suzukii* fungal infections and transmissions: A potential field suppression strategy** – Joan Cossentine, Mairi Robertson, Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Summerland, BC, Canada.
- 15:15 **23 Ant diseases: A neglected area in insect pathology?** – Roberto M. Pereira, Urban Entomology Lab., Entomology & Nematology Dept., University of Florida, Gainesville, FL, USA.
- 15:30 **24 The importance of searching for appropriate strains for the control of leaf-cutting ants** – Patricia J. Folgarait, Daniela Goffré, Laboratorio de Hormigas, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Bernal, Buenos Aires, Argentina.
- 16:00 – 16:30 **BREAK**
- Symposium Monday 16:30 – 18:30
Fungi/Microbial Control **2301**
- The (Underestimated) Value of Applied Research: Moving the Theoretical to the Practical**
- Moderators: Roma Gwynn, Michael Brownbridge, Travis Glare
- 16:30 **25 Why biopesticides sometimes fail** – Roma Gwynn¹, Michael Brownbridge², Travis Glare³, ¹Rationale Ltd., Lintlaw Farm Cottages, Duns TD113QA, Scotland; ²Vineland Research and Innovation Centre, Vineland Station, ON, Canada; ³Bio-Protection Research Centre, Lincoln University, Lincoln, New Zealand.
- 16:54 **26 Multiple roles so what should we measure? An ecological approach to promote the contribution of fungal entomopathogens in pest management within the agro-ecosystem** – Nicolai V. Meyling, Department of Plant and Environmental Sciences, University of Copenhagen, 1871 Frederiksberg C, Denmark.

Contributed Papers Monday 14:00 – 16:00

2311

Microbial Control 1

Moderator: Stefan Jaronski

- 17:18 **27 Research and development of biological crop protection products – bridging the gaps from discovery to field** – *Reed N. Royalty, Denise C. Manker*, Bayer CropScience, Biologics, 890 Embarcadero Drive, West Sacramento, CA, USA 95605.
- 17:42 **28 Adapting field trials for microorganisms – in practice** – *Edith Ladurner¹, Massimo Benuzzi¹, Sergio Franceschini²*, ¹CBC (Europe) S.r.l. – AREA TECNICA, via Calcinaro 2085 int. 7, 47521 Cesena (FC), Italy; ²CBC (Europe) S.r.l. – BIOGARD Division, via XXV Aprile 44, 24050 Grassobbio (BG), Italy.
- 18:06 **29 How do we improve efficacy monitoring of biopesticides?** – *Travis Glare¹, Michael Brownbridge², Roma Gwynn³*, ¹Bio-Protection Research Centre, Lincoln University, Lincoln, New Zealand; ²Vineland Research and Innovation Centre, Vineland Station, ON, Canada; ³Rationale Ltd., Lintlaw Farm Cottages, Duns TD113QA, Scotland.

- Canada, Saskatoon Research Centre, Saskatoon, SK, Canada.
- 17:45 **35-STU Wolbachia-mediated antiviral protection is life stage dependent** – *Aleksej L. Stevanovic, Pieter A. Arnold, Karyn N. Johnson*, School of Sciences, The University of Queensland, Brisbane 4072, Australia.
- 18:00 **36-STU A viral histone H4 modulates host gene expression by down regulating chromatin remodeling machinery** – *Sunil Kumar, Yonggyun Kim*, Department of Bioresource Sciences, Andong National University, Andong 760-749, Republic of Korea.
- 18:15 **37-STU Infection studies of an Agrotis segetum granulovirus isolate from Europe in cell cultures of AiE1611T** – *Gianpiero Gueli Alletti¹, Jörg T. Wennmann¹, Marina Eigenbrod², Eric B. Carstens^{1,2}, Regina G. Kleespies¹, Johannes A. Jehle¹*, ¹Julius Kühn Institute, Heinrichstr. 243, D-64287 Darmstadt, Germany; ²Queen's University, K7L 3N6 Kingston, ON, Canada.

Contributed Papers Monday 16:30 – 18:30
2306/9

Viruses 1

Moderators: Rollie Clem and Martin Erlandson

- 16:30 **30 Overexpression of two microRNAs enhances the infectivity of Sindbis virus TE5'2J strain in mosquitoes** – *Ning Huang¹, Jessica Moffitt¹, Kayvan Etebari², Sassan Asgari², Alexander W. E. Franz³, A. Lorena Passarelli¹, Rollie J. Clem¹*, ¹Division of Biology, Kansas State University, Manhattan, KS USA; ²School of Biological Sciences, University of Queensland, St. Lucia, Queensland, Australia; ³Department of Veterinary Pathology, University of Missouri, Columbia, MO USA.
- 16:45 **31 Microbial challenges and stress modify piRNA cluster abundance in the dengue mosquito, Aedes aegypti** – *Kayvan Etebari¹, Mazhar Hussain¹, Gregor J. Devine², Sassan Asgari²*, ¹Australian Infectious Disease Research Centre, School of Biological Sciences, The University of Queensland, Brisbane QLD 4072, Australia; ²QIMR Berghofer Institute of Medical Research, Brisbane, QLD 4006, Australia.
- 17:00 **32-STU A modified viral shuttle for exploring RNAi-related genes** – *David Neunemann¹, Heiko Vogel¹, David G. Heckel¹, Salvador Herrero², Ágata K. Jakubowska²*, ¹Entomology Department, Max Planck Institute for Chemical Ecology, Jena, Germany; ²Department of Genetics, Universitat de València, Burjassot, Spain.
- 17:15 **33-STU Role of dsRNA-mediated mechanism in the establishment of "latent" Glossina hytrosavirus infections in the tsetse fly** – *Irene K. Meki^{1,2}, Henry M. Kariithi³, Just M. Vlak¹, Adly M. M. Abd-Alla², Monique M. van Oers¹*, ¹Laboratory of Virology, Wageningen University, The Netherlands; ²Insect Pest Control Laboratory, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, Vienna, Austria; ³Biotechnology Research Institute, Kenya Agricultural & Livestock Research Organization, Kaptagat Rd, Loresho, Nairobi, Kenya.
- 17:30 **34-STU New target sites in pest control: Examining the role of REPAT proteins in defence against baculoviruses** – *Esenqül Ozdemir¹, Umut Toprak¹, Sibel Çavdar¹, Diana Bekkaoui², Dwayne Hegedus²*, ¹Ankara University, Faculty of Agriculture, Dept. of Plant Protection, Ankara, Turkey; ²Agriculture and Agri-Food

Contributed Papers Monday 16:30 – 17:30
2311

Microsporidia

Moderator: Yuliya Sokolova

- 16:30 **38 Host-pathogen interactions and genome evolution in two generalist and specialist microsporidian pathogens of mosquitoes** – *James J. Becnel¹, Christopher A. Desjardins², Neil Sanscrainte¹, Jonathan M. Goldberg², David Heiman², Sarah Young², Qiangdong Zeng², Hiten D. Madhani³, Christina A. Cuomo²*, ¹USDA, ARS, Center for Medical, Agricultural and Veterinary Entomology, 1600 SW 23rd Drive, Gainesville, Florida 32608, USA; ²Broad Institute of MIT and Harvard, Cambridge, Massachusetts 02142, USA; ³Department of Biochemistry and Biophysics, University of California-San Francisco, San Francisco, California 94158, USA.
- 16:45 **39 Ultrastructural analysis and SSU rRNA gene sequencing of Alfvénia sp. and Agglomerata cladocera from Siberian microcrustaceans shed light on diversification within the "Aquatic Outgroup"** – *Yuliya Y. Sokolova^{1,3}, Yuri S. Tokarev², Georgiy I. Rusakovich², Igor V. Senderskiy²*, ¹Institute of Cytology; ²Institute for Plant Protection, Russian Academy of Sciences, St.Petersburg, Russia, School of Veterinary Medicine, Louisiana State University, Baton Rouge LA, USA.
- 17:00 **40-STU Understanding the evolutionary loss of glycolysis in intranuclear crab microsporidians and the role of their highly mutated hexokinase in metabolism in their unusual habitat** – *Dominic Wiredu Bookye¹, Bryony Williams¹, Grant Stentiford², Thomas Williams³*, ¹College of Life and Environmental Sciences, University of Exeter, Geoffrey Pope. Stocker Road, Exeter. EX4 4QD; ²Centre of Environment, Fisheries and Aquaculture Science, CEFAS. The Nothe, Barrack Road, Weymouth, UK; ³Institute for Cell and Molecular Tyne Biosciences, University of Newcastle, Newcastle upon Tyne, Tyne and Wear, UK.
- 17:15 **41-STU Characterization of the spliceosome and large introns in microsporidia** – *Thomas A. Whelan, Cameron Grisdale, Naomi M. Fast*, Dept of Botany, University of British Columbia, Vancouver, BC, Canada.

SIP Division Business Meetings Monday 20:00-22:00

Microbial Control 2301
Diseases of Beneficial Invertebrates (+Workshop) 2306/9

Workshop Monday 20:00-22:00
Diseases of Beneficial Invertebrates 2306/9
Workshop
Environmental DNA

Organizers: Grant Stentiford and David Bass

TUESDAY – August 11

7:30 – 18:00 Information & Registration Outside Great Hall

Symposium Tuesday 8:00 – 10:00
Bacteria 2301

Mechanisms of Field Resistance to Bt Pesticides and Bt-crops

Moderator: Juan Luis Jurat-Fuentes

- 8:00 **42 Bt resistance in *Plutella* – too many trees?** – *Neil Crickmore*, School of Life Sciences, University of Sussex, Brighton, UK.
- 8:22 **43 Multiple resistance mechanisms selected in cabbage looper populations resistant to DiPel** – *Ping Wang*, Department of Entomology, Cornell University, New York State Agricultural Experimental Station, Geneva, NY, USA.
- 8:44 **44 Pink bollworm resistance to Bt cotton: Similar mechanisms in the lab and the field?** – *Jeffrey A. Fabrick¹, Jeyakumar Ponnuraj², Xianchun Li³, Yves Carrière³, Bruce E. Tabashnik²*, ¹USDA-ARS, U.S. Arid Land Agricultural Research Center, Maricopa, Arizona, USA; ²National Institute of Plant Health Management, Rajendranagar, Hyderabad, Andhra Pradesh, India; ³Department of Entomology, University of Arizona, Tucson, Arizona, USA.
- 9:06 **45 Mechanism of *Spodoptera frugiperda* resistance to Cry1Fa in Bt corn** – *Juan Luis Jurat-Fuentes¹, Siva R. K. Jakka¹, Rahul Banerje¹, James Hasler², Fangneng Huang³, Robert Meagher⁴, Rodney Nagoshi⁴*, ¹Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN, USA; ²DOW AgroSciences, Indianapolis, IN, USA; ³Department of Entomology, Louisiana State University AgCenter, Baton Rouge, LA, USA; ⁴USDA-ARS, Insect Behavior and Biocontrol Research Unit, Gainesville, FL, USA.
- 9:28 **46 Characterization of Cry3Bb1 resistance in western corn rootworm (*Diabrotica virgifera virgifera*)** – *Jeffrey A. Haas*, Monsanto Company, 700 Chesterfield Parkway West, Chesterfield, MO 63017, USA.
- 9:50 Discussion

Contributed Papers Tuesday 8:30 – 9:45
2311

Diseases of Beneficial Invertebrates 1

Moderators: David Bass and Helen Hesketh

8:30 **47 Pathogens on the horizon: Enhancing understanding of invasive alien entomopathogens and impacts on biodiversity** – *Helen Hesketh¹, Jørgen Eilenberg², Monique van Oers³, Helen Roy¹, Alison Dunn⁴, Grant Stentiford⁵, Beth Purse¹, Regina Kleespies⁶, Anja Amtoft-Wyns²*, ¹Centre for Ecology & Hydrology (CEH), Wallingford, United Kingdom; ²University of Copenhagen, Denmark; ³Wageningen University, Netherlands; ⁴University of Leeds, United Kingdom; ⁵Centre for Environment, Fisheries and Aquaculture Science (Cefas), United Kingdom; ⁶Julius Kühn-Institut, Federal Research Institute for Cultivated Plants, Germany.

8:45 **49 The prevalence of multi-host pathogens in *Bombus pascuorum* is influenced by honeybee domestication** – *Ivan Meeus¹, Laurian Parmentier¹, Jafar Maharramov¹, Niels Piot¹, Dirk C. de Graaf², Guy Smagghe¹*, ¹Department of Crop Protection, Ghent University, Coupure links 653, B-9000 Ghent, Belgium; ²Department of Biochemistry and microbiology, Ghent University, Ghent, Belgium.

9:00 **50-STU From the mosquito model to the bumblebee: A different behaviour of Vago mediated cross-talk between the small interfering RNA and Jak/stat pathways upon virus infection** – *Jinzhi Niu, Ivan Meeus, Guy Smagghe*, Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, Coupure links 653, B-9000 Ghent, Belgium.

9:15 **51 Variations in disease profile of juvenile edible crabs (*Cancer pagurus*) sampled from 3 different locations in the UK** – *Kelly S. Bateman¹, Stuart Ross¹, Rose Kerr¹, Ruth Hicks², Nick Taylor², Grant D. Stentiford¹*, ¹European Union Reference Laboratory for Crustacean Diseases, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Barrack Road, The Nothe, Weymouth, Dorset DT4 8UB, UK; ²Centre for Environment, Fisheries and Aquaculture Science (Cefas), Barrack Road, The Nothe, Weymouth, Dorset DT4 8UB, UK.

9:30 **52 Apicomplexan parasites cause regular mass mortalities in commercial scallop populations** – *Mark A. Freeman^{1,2}, Susan Inglis³, Árni Kristmundsson¹*, ¹Institute for Experimental Pathology at Keldur, University of Iceland, Reykjavik, Iceland; ²Institute of Ocean and Earth Sciences, University of Malaya, Kuala Lumpur, Malaysia; ³University of Massachusetts-Dartmouth, SMAST, Fairhaven, MA USA.

Contributed Papers Tuesday 8:00 – 10:00
2306/09

Viruses 2

Moderators: Eric Carstens and Monique van Oers

8:00 **53-STU AcMNPV encoded RING domain protein AC141 (Exon0) is associated with the GP64 and ME53 budding complex at the plasma membrane** – *Siddhartha Biswas¹, Yang Liu², Peter J. Krell², David A. Theilmann^{1,3}*, ¹Plant Science, Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC Canada V6T 1Z4; ²Department of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario, Canada N1G 2W1; ³Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Summerland, British Columbia, Canada.

8:15 **54 AcMNPV LEF-3 plays a critical role in both viral DNA replication and late gene expression** – *Mustapha El-Ayoubi, Eric B. Carstens*, Department of Biomedical and Molecular Sciences, Queen's University, Kingston,

- Ontario, Canada.
- 8:30 **55 The ODV-E66 of *Helicoverpa armigera* nucleopolyhedrovirus is involved in viral oral infection but is not essential for BV synthesis** – Dianhai Hou, Sijiani Luo, Fengqiao Zhou, Ranran Wang, Yanfang Zhang, Manli Wang, Hualin Wang, Zhihong Hu, Fei Deng, State Key laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, P.R. China.
- 8:45 **56 Phosphorylation induces structural changes in the *Autographa californica* nucleopolyhedrovirus P10 protein** – Linda A. King, Farheen Raza, Leo Graves, Sarah Irons, Robert D. Possee, Department of Biological and Medical Sciences, Oxford Brookes University, Oxford OX3 0BP UK.
- 9:00 **57-STU *Bombyx mori* nucleopolyhedrovirus ARIF-1 enhances systemic infection in *B. mori* larvae** – Ryuhei Kokusho, Toru Shimada, Susumu Katsuma, 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.
- 9:15 **58 Effect of me53 in baculovirus transcriptional regulation** – Yang Liu¹, Éva Nagy², Peter J. Krell¹, ¹Department of Molecular and Cellular Biology; ²Department of Pathobiology, University of Guelph, Guelph, ON, Canada.
- 9:30 **59 Termination of invertebrate iridescent virus mRNA transcripts: The role of a CATTa-containing hairpin** – İkbal Agah İnce^{1,2}, Monique M. van Oers¹, Gorben P. Pijlman¹, Just M. Vlak¹, ¹Laboratory of Virology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands; ²Medical Microbiology Dept., Acibadem University Medical School, Istanbul, Turkey.
- 9:45 **60 Post-translational modifications of the baculovirus protamine-like protein P6.9 and regulation of its hyperphosphorylation** – Ao Li, Haizhou Zhao, Qingying Lai, Zhihong Huang, Meijin Yuan, Kai Yang, State Key Laboratory of Biocontrol, Sun Yat-sen University, Guangzhou 510275, China.

10:00 – 10:30

BREAK

Contributed Papers Tuesday 10:30 – 11:30
2301

Bacteria 2

Moderators: Baltasar Escriche and Neil Crickmore

- 10:30 **61 Screening *Bacillus thuringiensis* strains with unapparent Crystals** – Changlong Shu¹, Xuewen Zhang^{1,2}, Neil Crickmore³, Fuping Song¹, Jie Zhang¹, ¹State Key Laboratory of Biology for Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, P. R. China; ²School of Life Science, Northeast Agricultural University, Harbin, P. R. China; ³School of Life Sciences, University of Sussex, Falmer, Brighton, UK.
- 10:45 **62-STU In search for novel bioactive molecules: Genome mining of the entomopathogenic bacterium *Photorhabdus luminescenssonorensis* (Gamma-Proteobacteria: Enterobacteriaceae)** – Rousef A. Orozco, S. Patricia Stock, Department of Entomology, University of Arizona, Tucson, AZ, USA.
- 11:00 **63 Structural mutants of the anti-feeding prophage** – Mark Hurst¹, Daria Rybakova², Alok Mitra³, ¹Innovative Farm systems, AgResearch, Lincoln Research Centre,

New Zealand; ²Institute of Environmental Biotechnology, Graz University of Technology, Austria; ³School of Biological Sciences, University of Auckland, Auckland, New Zealand.

- 11:15 **64 Loops replacements in domain II of Cry1Ab toxin with gut binding peptides alter insecticidal activity against the rice brown planthopper, *Nilaparvata lugens* (Stål)** – Shao Ensi¹, Sijun Liu², Lin Li³, Guan Xiong¹, ¹Key Laboratory of Biopesticide and Chemical Biology, Ministry of Education, Fujian Agriculture and Forestry University, 350002 Fuzhou, Fujian, PR China; ²Department of Entomology, Iowa State University, Ames, IA 50011, USA.

Contributed Papers

Tuesday 10:30 – 11:30

2311

Diseases of Beneficial Invertebrates 2

Moderators: David Bass and Helen Hesketh

- 10:30 **65-STU White spot syndrome virus (WSSV) infection: Understanding pathways of infection and opportunities for treatment from the perspective of a resistant species** – Bas Verbruggen, Lisa K. Bickley, Eduarda M. Santos, Charles R. Tyler, Grant D. Stentiford, Kelly S. Bateman, Ronny van Aerle, College of Life and Environmental Sciences, Exeter University, UK.
- 10:45 **66 Using genomics to identify host-pathogen interactions following white spot syndrome virus infection** – Lisa K. Bickley¹, Bas Verbruggen¹, Kelly S. Bateman², Grant D. Stentiford², Charles R. Tyler¹, Eduarda M. Santos¹, Ronny van Aerle², ¹College of Life and Environmental Sciences, Exeter University, UK; ²Centre for Environment, Fisheries and Aquaculture Science (CEFAS), Weymouth, UK.
- 11:00 **67 Effect of temperature on the immune, clinical and tissue response of American Lobster (*Homarus americanus*) experimentally infected with White Spot Syndrome Virus** – Louise-Marie D. Roux^{1,2,3}, Philip J. Byrne^{2,4}, K. Fraser Clark^{1,3}, Spencer J. Greenwood^{4,3}, Glenda M. Wright¹, Dorota W. Wadowska², ¹Department of Biomedical Sciences, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown PE, Canada; ²Gulf Biocontainment Unit, Department of Fisheries and Oceans Canada, Charlottetown, PE, Canada; ³AVC Lobster Science Centre, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown PE, Canada; ⁴Department of Pathology and Microbiology, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown PE, Canada; ⁵Electron Microscopy, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown PE, Canada.
- 11:15 **68 Paramyxa: Emergence of an enigmatic class of invertebrate parasites** – David Bass^{1,2}, Georgia Ward^{1,2}, Rose Kerr¹, Martyn Bennett¹, Rosaline Hulse², Grant D. Stentiford¹, ¹Centre for Environment, Fisheries, and Aquaculture Science (Cefas), Barrack Road, The Nothe, Weymouth, Dorset DT4 8UB, UK; ²Department of Life Sciences, The Natural History Museum, London, UK.
- 11:45 Buses leave for EXCURSIONS+BBQ Gage Residence
- 15:30 Buses leave for BBQ ONLY (for those not going on Excursions) Gage Residence
- 18:00 5k Run/Walk Cheakamus Centre
- 19:00 BBQ Cheakamus Centre
- 21:00 Buses return from BBQ to Gage Residence

WEDNESDAY – August 12

7:30 – 18:00 Information & Registration Outside Great Hall

Symposium Wednesday 8:00 – 10:00
Viruses **2301**

Advances in Host and Insect Virus Genomics

Moderators: David Theilmann and Martin Erlandson

- 8:00 **69 Macro- and micro-evolutionary trends in baculoviruses** – [Johannes A. Jehle](#), Laurin R. Monnheimer, Gianpiero Gueli Alletti, Jörg T. Wennmann, Institute for Biological Control, Federal Research Centre for Cultivated Plants, Julius Kühn Institute, Darmstadt, Germany.
- 8:24 **70 Alphabaculoviruses: Host Transcriptome Responses to Infection** – Yun-Ru Chen^{1,2}, Silin Zhong², Zhangjun Fei¹, Zhaofei Li³, Ping Wang⁴, Gary W. Blissard¹, ¹Boyce Thompson Institute at Cornell University, Ithaca, New York, USA; ²State Key Laboratory of Agrobiotechnology, The Chinese University of Hong Kong, Hong Kong, China; ³Key Laboratory for Applied Entomology, Northwest A&F University, Yangling, China; ⁴Department of Entomology, Cornell University, New York State Agricultural Experiment Station, Geneva, New York, USA.
- 8:48 **71 Polydnnaviruses: From discovery to current insights** – Michael R. Strand, Department of Entomology, University of Georgia, Athens, GA, USA.
- 9:12 **72 Dicitrovirus – hijacking the host translational machinery** – Eric Jan, Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, BC, Canada.
- 9:36 **73 Insect metagenomics-based discovery of novel, small RNA viral genomes** – Sijun Liu, Yuting Chen, Bryony C. Bonning, Department of Entomology, Iowa State University, Ames, IA, USA.

Contributed Papers Wednesday 8:00 – 9:45
2306/9

Microbial Control 2

Moderator: Travis Glare

- 8:00 **74 Potential of Aprehend™ (*Beauveria bassiana*) as a bed bug control agent** – Alexis M. Barbarin¹, Nina E. Jenkins², Giovani Bellicanta², Matthew B. Thomas^{2,3}, Coby Schal¹, ¹Department of Entomology, North Carolina State University, 100 Derieux Pl. Campus Box 7613, Raleigh, NC, 27695, USA; ²Department of Entomology, Penn State University, Merkle Lab University Park, PA 16802, USA; ³Center for Infectious Disease Dynamics, Penn State University, Merkle Lab, University Park, PA 16802, USA.
- 8:15 **75 *Beauveria bassiana* for the control of stored grain pests** – Aoife B. Dillon¹, Clare G. Storm¹, Freya C. L. Scoates², Adam J. Nunn¹, Sue E. Harris¹, Maureen E. Wakefield², Belinda Luke³, Bryony Taylor³, Dave Moore³, Pierre M. Grammare⁴, Olivier Potin⁴, ¹Exosect Ltd, Leylands Business Park, Winchester, Hampshire, SO21 1TH, UK; ²Food and Environment Research Agency, Sand Hutton, York, YO41 1LZ, UK; ³CABI Europe-UK, Bakeham Lane, Egham, Surrey, TW20 9TY, UK; ⁴Agrauxine, 18 route de Mauvières, Zi Tivoli, Loches, France 37600.

- 8:30 **76-STU Semi-field trials and tribulations** – Brian Lovett¹, Etienne Bilgo², Abdoulave Diabate², Raymond St. Leger¹, ¹University of Maryland, College Park; ²Centre Murax, Burkina Faso.
- 8:45 **77 A clean start strategy for seasonal poinsettia cuttings** – Michael Brownbridge¹, Rose Buitenhuis¹, Graeme Murphy², Taro Saito, Angela Brommit¹, ¹Vineland Research and Innovation Centre, 4890 Victoria Avenue North, Box 4000, Vineland Station, Ontario L0R 2E0, Canada; ²bioLogical Control Solutions, Welland, Ontario, Canada.
- 9:00 **78 Selection and characterization of Colombian entomopathogenic fungi against *Ceratomyxa tingomariana*** – Erika Grijalbo, Carlos Espinel, Adriana Santos, Cindy Mejia, Carolina Ruiz, Biological Control Laboratory, Colombian Corporation for Agriculture Research, CORPOICA. Mosquera, Colombia.
- 9:15 **79 Obstacles and opportunities for the use of biopesticides in conventional agriculture** – Surendra K. Dara, University of California Cooperative Extension, San Luis Obispo, CA, USA.
- 9:30 **80-STU Evaluation of the entomopathogenic fungus *Isaria sp.* as a biocontrol agent against pest insects in greenhouses** – Katharina Saar¹, Jasmin Philipp², Edgar Schliephake², Andreas Leclerque³, Manuel Werner¹, Johannes A. Jehle¹, Dietrich Stephan¹, ¹Institute for Biological Control, Julius Kühn Institute, Darmstadt, Germany; ²Institute for Resistance Research and Stress Tolerance, Julius Kühn Institute, Quedlinburg, Germany; ³Institute for Microbiology and Biochemistry, Geisenheim University, Geisenheim, Germany.

Contributed Papers Wednesday 8:00 – 9:45
2311

Nematodes 1

Moderator: Luis Leite

- 8:00 **81 Selection of lipid and protein sources for liquid fermentation of entomopathogenic nematodes** – Luis G. Leite¹, David I. Shapiro-Illan², Selcuk Hazir³, Mark A. Jackson⁴, ¹Instituto Biologico, APTA, CP 70, Campinas, SP 13001-970, Brazil; ²USDA-ARS, SEFTNRL, Byron, GA 31008, USA; ³Adnan Menderes University, Faculty of Arts and Sciences, Department of Biology, Aydin-Turkey; ⁴USDA-ARS, NCAUR, Peoria, IL, 61604, USA.
- 8:15 **82 An unsolvable root maze: Elevated atmospheric CO₂ increases root architectural complexity and reduces entomopathogenic nematode infectiousness** – A. Srikumar, L. Demarta, S. N. Johnson, L. Hiltbold, Hawkesbury Institute for the Environment, University of Western Sydney, Penrith, NSW, Australia.
- 8:30 **83-STU Investigating *Deladenus siricidicola* Kamona (Tylenchida: Neotylenchidae) to control *Sirex noctilio* (Hymenoptera: Siricidae) in North America** – Isis A. L. Caetano, Ann E. Hajek, Cornell University, Department of Entomology, Comstock Hall, 129 Garden Ave, Ithaca, New York, USA.
- 8:45 **84-STU Interactions between parasitic nematodes and bacteria in *Drosophila*** – M. A. Hanson, S. J. Perlman, Biology Department, University of Victoria, Victoria BC, Canada.
- 9:00 **85-STU Characterization of immune response genes in the parasitic nematode *Brugia malayi*** – Silvia Libro, Jeremy Foster, Barton Slatko, New England Biolabs,

- 240 County Road, Ipswich, MA, 01938, USA.
- 9:15 **86-STU One ring to bind them all: Is heme biosynthesis an influencing factor in *Wolbachia*-filarial nematode endosymbiosis?** – Ashley N. Luck, Jeremy M. Foster, Barton E. Slatko, Genome Biology Division, New England Biolabs, 240 County Road, Ipswich, MA, USA.
- 9:30 **87-STU Phenotypic diversity in the virulence of the entomopathogenic bacterium *Xenorhabdus bovienii* (Gamma-Proteobacteria: Enterobacteriaceae) reveals a type VI secretion system in its pan-genome** – John G. McMullen II¹, S. Patricia Stock², ¹School of Animal and Comparative Biomedical Sciences, The University of Arizona, Tucson, AZ, USA; ²Department of Entomology, The University of Arizona, Tucson, AZ, USA.

10:00 – 10:30

BREAK

Symposium Wednesday 10:30 – 12:30
Bacteria and Nematode **2306/9**

Intracellular Responses to Bacteria and Bacterial Toxins

Moderators: Raffi Aroian and Patrick Stock

- 10:30 **88 Insecticidal action, cellular interactions and response of combinations of *Photorhabdus*-Insect-related (Pir) and *Bacillus thuringiensis* Crystal (Cry) toxins** – Anaïs Castagnola¹, Rousel Orozco², Kathy Teng-Nelson³, Don Lightner³, Patricia Stock², ¹Center for Insect Science, University of Arizona, Tucson; ²Entomology Department, University of Arizona, Tucson; ³School of Animal and Comparative Biomedical Sciences, University of Arizona, Tucson, AZ, USA.
- 10:50 **89 Response to Cry1Ac intoxication in midgut cells of *Heliothis virescens* larvae** – Cris Oppert¹, Omalthage P. Perera², Thomas Lane¹, Margaret Staton¹, Heba M. Abdelgaffar¹, Juan Luis Jurat-Fuentes¹, ¹Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN, USA; ²USDA-ARS, Southern Insect Management Research Unit, Stoneville, MS, USA.
- 11:10 **90 Syringe-like injection mechanism of bacterial ABC toxins revealed in molecular detail** – Christos Gatsogiannis¹, Dominic Meusch¹, Alexander E. Lang², Klaus Aktories², Stefan Raunser¹, ¹Department of Structural Biochemistry, Max Planck Institute of Molecular Physiology, Dortmund, Germany; ²Institut für Experimentelle und Klinische Pharmakologie und Toxikologie, Albert-Ludwigs-Universität. Freiburg, Germany.
- 11:30 **91 *Caenorhabditis elegans* nck-1 plays a distinct and specific role in defense against bacterial pore-forming toxins** – Anand Sitaram, Yunqiang Yin, Raffi V. Aroian, University of Massachusetts Medical School, Worcester, MA, USA.
- 11:50 **92 The cell biology of *Wolbachia*-filarial nematode interactions and the dark side of symbiosis** – F. Landman^{1,2}, J. M. Foster³, M. L. Michalski⁴, B. E. Slatko³, W. Sullivan¹, ¹Department of Molecular, Cell and Developmental Biology, Sinsheimer Labs, University of California, Santa Cruz, California, USA; ²Centre de Recherche de Oshkosh, Biochimie Macromoléculaire, CNRS, Montpellier, France; ³Molecular Parasitology, New England Biolabs, Ipswich, Massachusetts, USA; ⁴Department of Biology and Microbiology, University of Wisconsin Oshkosh, Oshkosh, Wisconsin, USA.

- 12:10 **93 *Photorhabdus*: Light without heat?** – Alexia Hapeshi¹, Geraldine Mulley², Joe Healey¹, Andrew Millard, Nick Waterfield¹, ¹Warwick Medical School, Warwick University, Gibbet Hill, Coventry CV1 7AL, UK; ²School of Biological Sciences, University of Reading, Whiteknights, Reading, RG6 6AJ, UK.

Contributed Papers

Wednesday 10:30 – 12:30

2311**Fungi 1**

Moderators: Chad Keyser and Nicolai Meyling

- 10:30 **94 RNA-seq analysis reveals the potential antioxidant pathways regulated by multiprotein bridging factor 1 (BbMBF1) in the fungal entomopathogen *Beauveria bassiana*** – Xin-Ling Chu, Ming-Guang Feng, Sheng-Hua Ying, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, 310058, China.
- 10:45 **95-STU Unveiling a link of the Fus3 pathway to the biological control potential of *Beauveria bassiana*** – Zhi-Kang Wang, Ming-Guang Feng, Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou Zhejiang, 310058, China.
- 11:00 **96-STU Contributions of monothiol glutaredoxin and glutathione reductase to antioxidant capability and biological control potential of *Beauveria bassiana*** – Long-Bin Zhang, Li Tang, Ming-Guang Feng, Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, 310058, China.
- 11:15 **97-STU The Pal pathway regulates growth, conidiation, acidification, osmotic stress and virulence in *Beauveria bassiana*** – Jing Zhu, Ming-Guang Feng, Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, 310058, China.
- 11:30 **98-STU Regulatory role of a novel Ras GTPase (Ras 3) in conidiation, stress responses and virulence and its involvement in the HOG pathway of *Beauveria bassiana*** – Yi Guan, Ding-Yi Wang, Ming-Guang Feng, Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, 310058, China.
- 12:45 **99-STU A genome wide association study of resistance to *Metarhizium anisopliae*** – Jonathan B. Wang, Hsiao-Ling Lu, Raymond J. St. Leger, Entomology Department, University of Maryland, College Park, MD, USA.
- 12:00 **100-STU GeoChip analysis of the soil microbial community in turf and winter wheat treated with genetically modified *Metarhizium*** – Brian Lovett, Raymond St. Leger, University of Maryland, College Park, MD, USA.
- 12:15 **101 Diversity and distribution of *Metarhizium flavoviride* in agroecosystems: An overlooked entomopathogenic fungus?** – Chad A. Keyser, Henrik H. De Fine Licht, Bernhardt M. Steinwender, Nicolai V. Meyling, Department of Plant and Environmental Sciences, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg, Denmark.

Contributed Papers

Wednesday 10:30 – 12:30

2301**Viruses 3**

Moderator: Vera Ros

- 10:30 **102 Insecticidal parameters for the baculovirus infecting *Agrotis ipsilon* neonates** – *Robert W. Behle*, USDA-ARS-NCAUR, Crop Bioprotection Research Unit, Peoria, Illinois, USA.
- 10:45 **103 Haemocytes from *Crassostrea gigas* and OshV-1: A promising in vitro model to study host/virus interactions** – *Benjamin Morq¹*, *Nicole Faury¹*, *Tristan Renault²*, ¹Ifremer (Institut Français de Recherche pour l'Exploitation de la Mer) Laboratoire de Génétique et Pathologie des Mollusques Marins, La Tremblade, France; ²Ifremer Département Ressources Biologiques et Environnement, Nantes, France.
- 11:00 **104-STU Comparison of viral growth characteristic of two nucleopolyhedroviruses isolated from the genus *Adoxophyes*** – *Yasumasa Saito^{1,2}*, *Yasuhisa Kunimi¹*, *Maki N. Inoue²*, *Madoka Nakai¹*, ¹Laboratory of Biological Control, United Graduate School of Agricultural Science, Tokyo University of Agriculture and Technology, 3-5-8 Saiwai-cho, Fuchu, Tokyo 183-8509, Japan; ²Japan Society for the Promotion of Science.
- 11:15 **105-STU A novel type of resistance of the codling moth against *Cydia pomonella* granulovirus shows two different resistance mechanisms** – *Annette J. Sauer*, *Manuela M. Gebhardt*, *Eva Fritsch*, *Karin Undorf-Spahn*, *Johannes A. Jehle*, Insitute for Biological Control, Federal Research Center for Cultivated Plants, Julius Kühn Institute, Darmstadt, Germany.
- 11:30 **106-STU Nucleopolyhedrovirus and microsporidia in winter moth (*Operophtera brumata*) and bruce spanworm (*O. bruceata*) populations in the northeastern U.S.** – *Hannah J. Broadley¹*, *Joseph S. Elkinton¹*, *John P. Burand²*, *Matt Boucher²*, *Leellen F. Solter³*, ¹Organismic and Evolutionary Biology, University of Massachusetts, Amherst, MA, U.S.A.; ²Department of Microbiology, University of Massachusetts, Amherst, MA, U.S.A.; ³Department of Natural Resources and Environmental Sciences. University of Illinois, Champaign, IL, U.S.A.
- 11:45 **107-STU How climate and host behaviour influence nucleopolyhedrovirus infection dynamics in the western tent caterpillar** – *Paul R. MacDonald¹*, *Judith H. Myers²*, *Jenny S. Cory¹*, ¹Department of Biological Sciences, Simon Fraser University, Burnaby, Canada; ²Department of Zoology, University of British Columbia, Vancouver, Canada.
- 12:00 **108-STU The specialist baculovirus SeMNPV induces light-dependent tree-top disease in *Spodoptera exigua* caterpillars facilitated by the viral egt gene** – *Yue Han¹*, *Stineke van Houte²*, *Monique M. van Oers¹*, *Vera I.D. Ros¹*, ¹Laboratory of Virology, Wageningen University, Droevendaalsesteeg 1, 6708 PB, Wageningen, the Netherlands; ²Centre for Ecology and Conservation, Biosciences, University of Exeter, Penryn, Cornwall, UK.
- 12:15 **109 Baculovirus-induced tree-top disease revisited: The role of the egt gene** – *Vera I. D. Ros¹*, *Yue Han¹*, *Stineke van Houte^{1,2}*, *Monique M. van Oers¹*, ¹Laboratory of Virology, Wageningen University, Droevendaalsesteeg 1, 6708 PB, Wageningen, the Netherlands; ²Centre for Ecology and Conservation, Biosciences, University of Exeter, Penryn, Cornwall, United Kingdom.
- 12:30 – 14:00 **LUNCH** (on your own)

Workshop, with lunch Wednesday 12:30 – 14:00
(Students only) **2311**

Writing scientific articles: what reviewers, editors, and publishers are looking for

Dale Seaton, Leelen Solter

Symposium Wednesday 14:00 – 16:00
Fungus **2301**

Endophytic Entomopathogenic Fungi: “Pro-biotic” Microbial Associates of Plants?

Moderators: Don Roberts and Ray St. Leger

- 14:00 **110 Endophytic entomopathogenic fungi as “plant probiotics”: An important tool in protecting and promoting plant health?** – *Chad A. Keyser*, *Donald W. Roberts*, Department of Biology, Utah State University, Logan Utah, USA.
- 14:30 **111 Entomopathogenic fungi as endophytes: Interactions with host plants and herbivores** – *Stefan Vidal¹*, *Anant Patel²*, ¹Georg-August-University Goettingen, Department of Crop Sciences, Goettingen, Germany; ²University of Applied Sciences, Department of Engineering and Mathematics, Bielefeld, Germany.
- 15:00 **112 Trading insect nitrogen for photosynthate: Carbon translocation from a plant to an insect pathogenic, endophytic fungus** – *Michael J. Bidochka*, *Scott W. Behie*, Department of Biological Sciences, Brock University, St. Catharines, ON, Canada.
- 15:30 **113 *Metarhizium* as a multifactorial plant growth promoter** – *Raymond J. St. Leger*, Department of Entomology, University of Maryland, Maryland, USA.

Contributed Papers Wednesday 14:00 – 16:00
2306/9

Viruses 4

Moderators: Elisabeth Herniou and Madoka Nakai

- 14:00 **114 Population structure of *Spodoptera litura* multicapsid nucleopolyhedroviruses from Pakistan** – *Ghulam Ali^{1,2}*, *Marleen Henkens²*, *Elio Schijlen³*, *Wopke van der Werf⁴*, *Just M. Vlak²*, ¹CABI Rawalpindi, Pakistan; ²Laboratory of Virology, Wageningen University, Wageningen, The Netherlands; ³Bioscience, Wageningen UR, Wageningen, The Netherlands; ⁴Centre for Crop Systems Analysis, Wageningen University, Wageningen, The Netherlands.
- 14:15 **115 Genome sequence and environmental tolerance of a granulovirus mutant that produces abnormally large occlusion bodies** – *Madoka Nakai¹*, *Robert L. Harrison²*, *Haruaki Uchida¹*, *Yasuhisa Kunimi¹*, ¹Tokyo University of Agriculture and Technology, Saiwai, Fuchu, Tokyo 183-8509, Japan; ²Invasive Insect Biocontrol and Behavior Laboratory, USDA Agricultural Research Service (USDA-ARS), Beltsville Agricultural Research Center, 10300 Baltimore Avenue, Beltsville, MD 20705, USA.
- 14:30 **116 The complete genome of *Aedes sollicitans* nucleopolyhedrovirus** – *Omaththage P. Perera¹*, *James J. Becnel²*, ¹Southern Insect Management Research Unit, USDA-ARS, Stoneville, MS 38776; ²Mosquito and Fly Research Unit, CMAVE, USDA-ARS, Gainesville, FL, USA.

- 14:45 **117-STU Lake Sinai Virus (LSV) diversity in Hymenoptera** – Diane Bigot¹, Elisabeth A. Herniou¹, Nicolas Galtier², Philippe Gayral¹, ¹Institut de Recherche sur la Biologie de l’Insecte, UMR 7261, CNRS, Université François-Rabelais, 37200 Tours, France; ²Institut des Sciences de l’Evolution de Montpellier, UMR 5554, CNRS, Université Montpellier 2, Place E. Bataillon, 34095 Montpellier, France.
- 15:00 **118 Genomic landscape of AcMNPV adaptation** – Aurélien Chateigner, Cindy Pontleve, Carole Labrousse, Elisabeth A. Herniou, Institut de Recherche sur la Biologie de l’Insecte, UMR CNRS 7261, Université François Rabelais de Tours, Faculté des Sciences et Techniques, Avenue Monge - Parc Grandmont, 37200 Tours, France.
- 15:15 **119 In search of the ancestor of banchine polydnaviruses** – Catherine Béliveau¹, Alejandro Cohen², Don Stewart¹, Georges Periquet³, Abdelmadjid Djoumad¹, Lisa Kuhn⁴, Don Stoltz⁴, Brian Boyle⁵, Anne-Nathalie Volkoff⁶, Elisabeth A. Herniou³, Jean-Michel Drezen³, Michel Cusson¹, ¹Natural Resources Canada, Canadian Forest Service, Laurentian Forestry Centre, Quebec City, Quebec, Canada; ²Proteomics Core Facility, Dalhousie University, Halifax, Nova Scotia, Canada; ³Institut de Recherche sur la Biologie de l’Insecte (IRBI), Université François-Rabelais et CNRS UMR 7261, Tours, France; ⁴Department of Microbiology and Immunology, Dalhousie University, Halifax, Nova Scotia, Canada; ⁵Institut de Biologie Intégrative et des Systèmes, Université Laval, Quebec City, Quebec, Canada; ⁶UMR 1333 INRA, Université Montpellier, "Diversité, Génomes, Interactions Microorganismes-Insectes" (DGIMI), Montpellier, France.
- 15:30 **120 Construction and rescue of a synthetic baculovirus, AcMNPV-WIV-Syn1.0, that retains the properties of the parental virus** – Yu Shang, Fei Deng, Gengfu Xiao, Dianhai Hou, Kai Pan, Manli Wang, Hualin Wang, Zhihong Hu, State Key laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, P.R. China.
- 15:45 **121 Generating a host ranges extended recombinant baculovirus** – Tzonqyuan Wu, Chao-Yi Teng, Mean-Shine Chen, Department of Bioscience Technology, Chung Yuan Christian University, Chung Li, Taiwan.

Workshop Wednesday 14:00 – 15:00
Nematodes **2311**

Nematodes Workshop

Moderators: David Shapiro-Ilan and Harry Kaya

- 14:00 **122 Entomopathogenic nematode species in insect pest suppression: Can their use be optimized by proper application approaches?** – Harry K. Kaya¹, Selcuk Hazir², David Shapiro-Ilan³, ¹Department of Entomology and Nematology, University of California, Davis, CA 95616, USA; ²Department of Biology, Faculty of Arts and Sciences, Adnan Menderes University, Aydin, TURKEY; ³USDA-ARS, SE Fruit and Tree Nut Research Laboratory, Byron, GA 31008, USA.
- 14:15 **123 EPNs from lab to field against insect pests of fine turfgrass: Overcoming obstacles in research and implementation** – Albrecht M. Koppenhöfer¹, Olga S. Kostromytska¹, Shaohui Wu¹, Benjamin A. McGraw², Lemma Ebssa³, ¹Department of Entomology, Rutgers University, New Brunswick, NJ 08901, USA; ²Department

of Plant Science, Pennsylvania State University, University Park, PA, 16802, USA; ³Institute for Health, Health Care Policy and Aging Research, Rutgers University, New Brunswick, NJ 08901, USA.

- 14:30 **124 Plant scream, insect succumb: From concepts to applications** – J. Hiltbold, Hawkesbury Institute for the Environment, University of Western Sydney, Penrith, NSW, Australia.
- 14:45 Discussion

16:00 – 16:30

BREAK

Wednesday 16:30 – 18:30
Great Hall

POSTERS

Bacteria

- BA-1-STU Novel mosquitocidal activity of strains LBIT 980 and LBIT-1217 of *Bacillus thuringiensis*** – Mariana Fernández-Navarro¹, Cristina Del Rincón-Castro², Jorge E. Ibarra¹, ¹Department of Biotechnology and Biochemistry, CINVESTAV-Irapuato, Gto. Mexico; ²Life Sciences Division, University of Guanajuato, Irapuato, Gto. Mexico.
- BA-2-STU Inoculation and translocation of the spore-crystal complex from *Bacillus thuringiensis* in vascular tissue of bean plants** – Rosalina García-Suárez¹, Cristina Del Rincón-Castro², Jorge E. Ibarra¹, ¹Department of Biotechnology and Biochemistry, CINVESTAV-Irapuato, Gto. Mexico; ²Life Sciences Division, University of Guanajuato, Irapuato, Gto. Mexico.
- BA-3-STU Diversity of Bacteria Composition of Spined Soldier Bug, *Podisus maculiventris* (Hemiptera: Pentatomidae) Digestive System** – E. M. Romaqno¹, C. A. Dunlap², L. B. Flor-Weiler², T. A. Coudron³, A. P. Rooney², ¹“Luiz de Queiroz” College of Agriculture, University of Sao Paulo, Piracicaba, Sao Paulo, 13418900, Brazil; ²National Center for Agricultural Utilization Research, Agricultural Research Service, U.S. Department of Agricultural, Peoria, IL, 61604, USA; ³Biological Control of Insects Research Service, U.S. Department of Agricultural, Columbia, MO, USA.
- BA-4-STU Use of NGS to assess the effect of antibiotics on the bacterial community of insects** – Diana Wilches^{1,2}, Kevin D. Floate², Paul Coghlin², ¹University of Lethbridge; ²Agriculture and Agri-Food Canada, Research Centre, Lethbridge, AB, Canada.
- BA-5 Status of resistance to *Bt* cotton in China: Cotton bollworm and Pink bollworm** – Yulin Gao¹, Chenxi Liu¹, Yidong Wu², Kongming Wu¹, ¹State Key Laboratory for Biology of Plant Disease and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, PR China; ²Department of Entomology, College of Plant Protection, Nanjing Agricultural University, Nanjing 210095, PR China.
- BA-6 Evidence of field-evolved resistance of *Spodoptera frugiperda* to *Bt* corn expressing Cry1F in Brazil that is still sensitive to modified *Bt* toxin** – Anabele A. Lima^{1,5}, Rose Monnerat², Erica Martins², Cristina

- Macedo^{1,3}, Paulo Queiroz², Lílian Praça¹, Marcelo C. Soares², Helio Moreira¹, Isabella Grisi¹, Joseane Silva¹, Mario Soberon⁴, Alejandra Bravo⁴, Embrapa Recursos Genéticos e Biotecnologia, Brasília, Distrito Federal, Brazil; ²Instituto Mato-Grossense do Algodão, Cuiabá, Mato Grosso, Brasília, Brasília, Distrito Federal, Brazil; ³Departamento de Microbiologia, Universidade de Brasília, Brasília, Distrito Federal, Brazil; ⁴Instituto de Biotecnologia, Universidad Nacional Autónoma de México, Cuernavaca, Morelos, Mexico; ⁵University Center of Brasília, Distrito Federal, Brazil.
- BA-7 Behavioural feeding responses of susceptible and resistant *Trichoplusia ni* larvae to *Bacillus thuringiensis* – Alida F. Janmaat, Mandy Gelderman,, Martin Muermann, Biology Department, University of the Fraser Valley, Abbotsford, BC, Canada.**
- BA-8 Discovering novel *Bt* toxins for better bio-pesticides and biotech crops – Jim X. J. Fang^{1,2}, W. F. Zhang³, Y. Zhou², P. T. Gong², ¹The HITAR Institute Canada Inc., Richmond, British Columbia, Canada; ²Hainan Institute of Tropical Agricultural Resources, Sanya, Hainan, China; ³College of Life Sciences, Hainan Normal University, Haikou, Hainan, China.**
- BA-9 Toxicity and interaction of Cry1 proteins from *Bacillus thuringiensis* in *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) – Manoel Victor F. Lemos¹, Isis Sebastião¹, Camila S. Figueiredo¹, Ana Rita N. Lemes¹, Ricardo A. Polanczyk², Janete A. Desidério¹, Faculdade de Ciências Agrárias e Veterinárias, UNESP – Univ. Estadual Paulista Júlio de Mesquita Filho; ¹Departamento de Biologia Aplicada à Agropecuária; ²Departamento de Fitossanidade. Rod. Prof. Paulo Donato Castellane, km. 5, CEP14884-900, Jaboticabal, São Paulo, Brasil.**
- BA-10 Interaction of Vip3Aa42 and Cry11a10 proteins from *Bacillus thuringiensis* and toxicity to *Anticarsia gemmatilis* (Hübner, 1818) (Lepidoptera: Erebidae) – Suzana C. Marucci, Janete A. Desidério, Manoel Victor F. Lemos, Departamento de Biologia Aplicada à Agropecuária, Univ. Estadual Paulista Júlio de Mesquita Filho, UNESP/Campus de Jaboticabal, SP, CEP 14884-900, Brasil.**
- BA-11-STU Parallels between the Cry41Aa Parasporin and insecticidal *Bt* toxins – Barbara Domanska, Michelle West, Neil Crickmore, Department of Biochemistry, School of Life Sciences, University of Sussex, Falmer, Brighton, BN1 9QG, UK.**
- BA-12-STU Unusual pore formation by a new *Bacillus thuringiensis* toxin – Eva Forte Verdejo¹, Vincent Lemieux^{1,2}, Léna Potvin¹, Timothy Hey³, Xiaoping Xu³, Samantha Griffin³, Vimbai Chikwana³, David McCaskill³, Ken Narva³, Vincent Vachon¹, Jean-Louis Schwartz^{1,4}, ¹Département de physiologie moléculaire et intégrative and Groupe d'étude des protéines membranaires, Université de Montréal, Montréal, QC, Canada; ²Département de biologie, Université de Sherbrooke, Sherbrooke, QC, Canada; ³Dow Agrosiences LLC, Indianapolis (IN 46268), IN, USA.; ⁴Centre SÈVE de recherche en sciences du végétal, Université de Sherbrooke, Sherbrooke, QC, Canada.**
- BA-13-STU Construction & characterization of novel *Bacillus thuringiensis* Cry1-type genes with improved insecticidal activities – Jong Hoon Kim¹, Jae Young Choi², Pang Ying¹, Ha Kyu Baik¹, Seok Hee Lee¹, Woo Jin Kim², Yeon Ho Je¹, ¹Department of Agricultural**
- Biotechnology, Seoul National University, Seoul, Republic of Korea; ²Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul, Republic of Korea.
- BA-14-STU Development of a Cry toxin activity-improving method based on the directed evolution that targets ABCC2 – Kazuhiro Imamura¹, Natsuko Nakajima¹, Haruka Endo^{1,2}, Ryoichi Sato¹, ¹Graduate School of Bio-Applications and Systems Engineering Tokyo University Of Agriculture and Technology, Koganei, Tokyo, Japan; ²Japan Society for the Promotion of Science Research Fellowship for Young Scientists.**
- BA-15 Carbohydrate binding domain of Vip3Aa is involved in receptor binding – Kun Jiang¹, Yu Yuan¹, Dong-hui Hua¹, Tingting Wang, Yue-hua Chen^{1,2,3}, Jun Cai^{1,2,3}, ¹Department of Microbiology, College of Life Sciences, Nankai University, Tianjin, China; ²Key Laboratory of Molecular Microbiology and Technology, Ministry of Education, Tianjin, China; ³Tianjin Key Laboratory of Microbial Functional Genomics, Tianjin, China.**
- BA-16 Insecticidal spectrum and mode of action of the *Bacillus thuringiensis* Vip3Ca insecticidal protein – Joaquín Gomis-Cebolla¹, Iñigo Ruiz de Escudero^{2,3}, Maissa Chakroun¹, Natalia Mara Vera-Velasco¹, Patricia Hernández-Martínez¹, Carmen Sara Hernández-Rodríguez¹, Yolanda Bel¹, Baltasar Escriche¹, Primitivo Caballero^{2,3}, Juan Ferré¹, ¹ERI de Biotecnología y Biomedicina (BIOTECMED), Universitat de València, Burjassot, Spain; ²Instituto de Agrobiotecnología, CSIC-UPNA, Gobierno de Navarra, Campus Arrosadía, Navarra, Spain; ³Laboratorio de Entomología Agrícola y Patología de Insectos, Universidad Pública de Navarra, Pamplona, Spain.**
- BA-17 Binding to brush border vesicles and midgut processing of *Bacillus thuringiensis* Vip3Aa toxin to *Helicoverpa armigera* susceptible and Vip3Aa-resistant insects – Maissa Chakroun¹, Nuria Banyuls¹, Tom Walsh², Sharon Downes³, Rod Mahon³, Bill James², Juan Ferré¹, ¹ERI of Biotechnology and Biomedicine (BIOTECMED), Universitat de València, Burjassot, Spain; ²Land and Water Flagship, CSIRO, Canberra ACT 2601; ³Agriculture Flagship, CSIRO, Narrabri NSW 2390.**
- BA-18 Proteolytic processing of *Bacillus thuringiensis* Cry3 proteins by gut proteases and binding to brush border membrane vesicles from *Cylas puncticolis* (Brentidae) – Natalia M. Vera-Velasco, Patricia Hernández-Martínez, Baltasar Escriche, Departamento de Genética, Facultad de CC. Biológicas, Universitat de València, Spain.**
- BA-19 Domain III of *Bacillus thuringiensis* Cry1Ie toxin contributes to its binding to peritrophic membrane of Asian corn borer – Dongmei Feng, Zhen Chen, Nan Zhang, Chunlu Zhang, Shuyuan Guo, School of Life Science, Beijing Institute of Technology, Beijing 100081, China.**
- BA-20-STU Loop regions of domain II of Cry1Aa have an important role for binding with BmABCC2 and its BmABCC2-dependent activity – Satomi Adegawa¹, Shiho Tanaka^{1,2}, Shingo Kikuta¹, Ryoichi Sato¹, ¹Graduate School of Bio-Applications and Systems Engineering Tokyo University of Agriculture and Technology, Koganei, Tokyo, Japan; ²Japan Society for the Promotion of Science Research Fellowship for Young Scientists.**

- BA-21** **sRNA mediated Cry toxin gene silencing helps *Bacillus thuringiensis* evading nematode feeding cessation defense behavior** – Xiaoxia Luo, Donghai Peng, Ni Zhang, Suxia Guo, Jinshui Zheng, Ling Chen, Jian Lin, Lifang Ruan, Ming Sun, State Key Laboratory of Agricultural Microbiology, College of Life Science and Technology, Huazhong Agricultural University, Wuhan 430070, Hubei, China.
- BA-22** **Characterization of *Wolbachia* endosymbiont of *Lygus lineolaris*** – Omaththage P. Perera, Gordon L. Snodgrass, Randall G. Luttrell, Southern Insect Management Research Unit, USDA-ARS, Stoneville, MS 38776, USA.
- BA-23** **Biochemical characterization of parasporin-4 and effects of the pro-parasporin-4 diet on the health of mice** – Shiro Okumura¹, Hironori Koga², Kuniyo Inouye^{3,4}, Eiichi Mizuki¹, ¹Biotechnology and Food Research Institute, Fukuoka Industrial Technology Center, Fukuoka, Japan; ²Division of Gastroenterology, Department of Medicine, Kurume University School of Medicine, Fukuoka, Japan; ³Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Kyoto, Japan; ⁴Inouye Laboratory of Enzyme Chemistry (iLEC), Kyoto, Japan.

Diseases of Beneficial Invertebrates

- DB-1-STU** **Chitin-degrading protein PICBP49 - a key virulence factor of *Paenibacillus larvae*** – Henriette Knispel¹, Anne Fünfhaus¹, Eva Garcia-Gonzalez¹, Lena Poppinga¹, Jennifer S M Loose², Gustav Vaaje Kolstad², Elke Genersch¹, ¹Institute for Bee Research, Hohen Neuendorf, Division of Molecular Microbiology and Bee Pathology, Friedrich-Engels-Str.32, D-16540 Hohen Neuendorf, Germany; ²Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, P.O. Box 5003, N-1432 Ås, Norway.
- DB-2-STU** **Characterization of virulence factors in the fatal honey bee disease American foulbrood caused by *Paenibacillus larvae*** – Julia Ebeling, Lena Poppinga, Anne Fünfhaus, Elke Genersch, Institute for Bee Research, Hohen Neuendorf, Brandenburg, Germany.
- DB-3** **Decentralised molecular diagnostics and remote data reporting for management of disease in global aquaculture** – Kelly S. Bateman¹, Michelle Pond¹, Peter Munday², Olga Gandelman², Grant D. Stentiford¹, ¹European Union Reference Laboratory for Crustacean Diseases, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Barrack Road, The Nothe, Weymouth, Dorset DT4 8UB, UK; ²Epistem, Genedrive, Epistem Ltd., 48 Grafton Street, Manchester, M13 9XX, UK.
- DB-4** **Virus prevalence in bee populations in apple orchards in New York, USA** – John P. Burand¹, Shuning Zheng², Bryan N. Danforth³, ¹Department of Microbiology; ²Graduate Program in Molecular and Cellular Biology, University of Massachusetts - Amherst, Amherst, MA 01003, USA; ³Department of Entomology Cornell University, Ithaca, NY 14853 USA.
- DB-5** **Insect pathological challenges in insects produced for food and feed** – Jørgen Eilenberg¹, Christina Nielsen-LeRoux², Silvia Cappellozza³, Just M. Vlask⁴, Annette B. Jensen¹, ¹University of Copenhagen, Department of Plant and Environmental Sciences, Copenhagen, Denmark; ²INRA, UMR 1319 Micalis-AgroParisTech, France; ³Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Honeybee and Silkworm Research Unit, Padova, Italy; ⁴Wageningen University, Laboratory of Virology, Wageningen, the Netherlands.
- DB-6-STU** **The influences of insect-mediated mental healthcare program to children with mental disease** – Seung Hee Lee¹, Sung Min Bae¹, Young Soon Jun², Tae Young Shin¹, Yong Oh Ahn¹, Won Seok Gwak¹, Soo Dong Woo¹, ¹Department of Agricultural Biology, College of Agriculture, Life & Environment Science, Chungbuk National University, Cheongju 361-763, Korea; ²Department of Neuropsychiatry, Konkuk University Chungju Hospital, Chungju 380-704, Korea.
- DB-7** **Development and efficacy of insect-mediated mental healthcare program for the public** – Sung Min Bae¹, Young Soon Jun², Tae Young Shin¹, Won Seok Gwak¹, Yong Oh Ahn¹, Seung Hee Lee¹, Soo Dong Woo¹, ¹Department of Agricultural Biology, College of Agriculture, Life & Environment Science, Chungbuk National University, Cheongju 361-763, Korea; ²Department of Neuropsychiatry, Konkuk University Chungju Hospital, Chungju 380-704, Korea.
- DB-8-STU** **Efficacy of insect-mediated mental healthcare program to an adolescent** – Won Seok Gwak¹, Tae Young Shin¹, Young Soon Jun², Sung Min Bae¹, Yong Oh Ahn¹, Seung Hee Lee¹, Soo Dong Woo¹, ¹Department of Agricultural Biology, College of Agriculture, Life & Environment Science, Chungbuk National University, Cheongju 361-763, Korea; ²Department of Neuropsychiatry, Konkuk University Chungju Hospital, Chungju 380-704, Korea.

Fungi

- FU-1** **ITS region analysis of isolates of *Beauveria bassiana*, a pathogenic fungus to the silkworm, *Bombyx mori* L.** – Liangen Shi, Jie Jin, College of Animal Sciences, Zhejiang University, Hangzhou, Zhejiang, P.R.China.
- FU-2** **Molecular characterization of *Isaria fumosorosea* from citrus and strawberry crops in Brazil** – Celeste P. D'Alessandro¹, Ricardo Macedo do Nascimento¹, Gustavo Maruyama Mori^{2,3}, Maria I. Zucchi³, Italo Delalibera Junior¹, ¹Department of Entomology and Acarology, "Luiz de Queiroz" College of Agriculture (ESALQ), University of São Paulo (USP), Piracicaba, São Paulo, Brazil; ²Center for Molecular Biology and Genetic Engineering, University of Campinas, Campinas, São Paulo, Brazil; ³São Paulo Agency for Agribusiness Technology, Piracicaba, São Paulo, Brazil.
- FU-3-STU** **Comparative genomics of cold-adapted *Metarhizium frigidum*** – Brian Lovett, Raymond St. Leger, University of Maryland, College Park, USA.
- FU-4** **Identification of *Drosophila* mutants affecting defense to an entomopathogenic fungus** – Hsiao-Ling Lu, Jonathan Wang, Markus Brown, Chris Euerle, Raymond St. Leger, Entomology Department, University of Maryland, College Park, MD, USA.
- FU-5-STU** **Persistence of two Brazilian isolates of *Metarhizium* in a strawberry cropping system using microsatellite markers** – Thiago Rodrigues de Castro^{1,2}, Johanna Mayerhofer³, Jürg Enkerli³, Jørgen Eilenberg², Nicolai

- V. Meyling², Italo Delalibera Jr.¹, ¹University of São Paulo (ESALQ), Brazil; ²Department of Plant and Environmental Sciences, University of Copenhagen, Denmark; ³Institute for Sustainability Sciences, Agroscope, Switzerland.
- FU-6** **Susceptibility of biocontrol fungi in the genera *Nomuraea*, *Isaria*, *Purpureocillium*, *Pochonia*, and *Trichoderma* to imbibitional damage and its mitigation through increased conidial quality** – Marcos Faria, Irene Martins, Daniela A. Souza, Rogério B. Lopes, EMBRAPA Genetic Resources and Biotechnology, Brasília, DF, Brazil.
- FU-7-STU** **Light during mycelial growth induces increased tolerance of conidia to different types of stress in entomopathogenic fungi** – Luciana P. Dias^{1,2}, Claudinéia A. S. Araujo¹, Paulo C. Ferreira¹, Breno Pupin¹, Drauzio E. N. Rangel¹, ¹Instituto de Pesquisa e Desenvolvimento, Universidade do Vale do Paraíba, São José dos Campos, SP 12244-000, Brazil; ²Escola de Engenharia de Lorena (EEL/USP)/ Pós-Graduação em Biotecnologia Industrial, Estrada Municipal do Campinho, Lorena, SP, Brazil.
- FU-8** **The great soil-sampling survey: A Utah State University/USDA collaborative project to find new entomopathogenic fungi from western USA soil** – Chad A. Keyser, Samuel P. Sherwood, Everton K. K. Fernandes, Carmina Moore, Patricia S. Golo, JoAnn Stark, Alesandra M. Fernandes, Rodrigo B. Ferreira, Scott Treat, Fabiana Alvarez, Janitha B. Nandalochana, Holly C. Suisse, Stefanie Selamet, Laurel Nicolson, Skylar Christensen, R. Nelson Foster*, Lerry E. Jech*, Stefan T. Jaronski*, Donald W. Roberts, Department of Biology, Utah State University, Logan Utah, USA. *USDA, APHIS, PPQ, CPHST.
- FU-9** **Comparison of the use of insect baiting methods versus selective media when determining the diversity of *Metarhizium* spp. in soil** – Carmela Hernández-Domínguez, Ariel W. Guzmán-Franco, Postgrado en Fitosanidad-Entomología y Acarología, Colegio de Postgraduados, Km. 36.5 Carretera México-Tezcoco, Montecillo, Tezcoco, Estado de Mexico, 56230, Mexico.
- FU-10-STU** **Antifungal activity of entomopathogenic fungi isolated in Korea and their geographical characteristics** – Yong Oh Ahn, Tae Young Shin, Sung Min Bae, Won Seok Gwak, Seung Hee Lee, Soo Dong Woo, Department of Agricultural Biology, College of Agriculture, Life & Environment Science, Chungbuk National University, Cheongju 361-763, Korea.
- FU-11** **Characterization and pathogenicity of a *Beauveria pseudobassiana* strain that collapsed a laboratory colony of *Dendroctonus ponderosae* (Coleoptera: Scolytidae)** – George Kyei-Poku¹, Debbie Gauthier¹, Fernando Mantouvan¹, Johny Shajahan², Will Fick¹, ¹Natural Resources Canada, Canadian Forest Service, Great Lakes Forestry Centre Sault Ste. Marie, Ontario, Canada; ²Amco Farms Inc., Leamington, ON, Canada.
- FU-12** **Entomopathogenic fungi for six species of leaf-cutting ants from different sites of Argentina** – Daniela Goffré, Jorge A. Marfetan, Patricia J. Folgarait, Laboratorio de Hormigas, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Bernal, Buenos Aires, Argentina.
- FU-13** **First Isolation of *Beauveria* and *Metarhizium* from a wheat stem borer, *Cephus cinctus* (Hymenoptera: Cephidae) in North America** – Stefan T. Jaronski¹, Gadi V. P. Reddy², Shaohui Wu², John F. Gaskin¹, Stephen Rehner³, ¹Agricultural Research Service, U.S. Department of Agriculture, Northern Plains Agricultural Research Laboratory, Sidney MT 50270 USA; ²Montana State University, Western Triangle Research Center, Conrad MT 59425 USA; ³Agricultural Research Service, U.S. Department of Agriculture, Systematic Mycology Laboratory, Beltsville MD 20705 USA.
- FU-13** **First report of a mosquito-pathogenic *Leptolegnia* sp. (Saprolegniales) in Brazil** – Cristian Montalva¹, Richard A. Humber², Karine dos Santos¹, Stefanie Buchter¹, Karin Collier³, Luiz F.N. Rocha^{1,4}, Éverton K. K. Fernandes¹, Christian Luz¹, ¹Laboratório de Patologia de Invertebrados, Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, GO, Brasil; ²USDA-ARS Collection of Entomopathogenic Fungal Cultures, RW Holley Center for Agriculture and Health, Ithaca, NY, USA; ³Centro Universitário UNIRG, Gurupí, TO, Brasil; ⁴Instituto Federal de Educação, Ciência e Tecnologia de Goiás, Aparecida de Goiânia, GO, Brasil.
- FU-15** **The puzzle of identifying mosquito-pathogenic isolates of *Leptolegnia*** – Richard A. Humber¹, Cristian Montalva², Christian Luz², ¹USDA-ARS Collection of Entomopathogenic Fungal Cultures, RW Holley Center for Agriculture and Health, Ithaca, NY, USA; ²Laboratório de Patologia de Invertebrados, Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, GO, Brasil.
- FU-16** **A treasure among the trash: *Pandora bullata* from a Brazilian garbage dump** – Richard A. Humber¹, Cristian Montalva², Karin F. S. Collier³, Christian Luz², ¹USDA-ARS Collection of Entomopathogenic Fungal Cultures, RW Holley Center for Agriculture and Health, Ithaca, NY, USA; ²Laboratório de Patologia de Invertebrados, Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, GO, Brasil; ³Centro Universitário UNIRG, PROPEQS, Gurupí, TO, Brasil.
- FU-17-STU** **Effects of temperature on germination, growth, and sporulation of *Culicinomyces* species** – Juscelino Rodrigues¹, Richard A. Humber², Éverton K. K. Fernandes¹, Christian Luz², ¹Laboratório de Patologia de Invertebrados, Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, GO, Brasil; ²USDA-ARS Collection of Entomopathogenic Fungal Cultures, RW Holley Center for Agriculture and Health, Ithaca, NY, USA.
- FU-18-STU** ***Beauveria bassiana* inhibits host seeking behavior of *Anopheles stephensi*** – Minehiro Ishii^{1,2,3}, Masanori Koike³, Daigo Aiuchi⁴, ¹The United Graduate School of Agricultural Sciences, Iwate University, Japan; ²Research Fellowship for Young Scientists, Japan Society for the Promotion of Science; ³Department of Agro-environmental Science, Obihiro University of Agriculture & Veterinary Medicine, Japan; ⁴Research Center for Global Agro-medicine, Obihiro University of Agriculture & Veterinary Medicine, Japan.
- FU-19** **Biological activity of entomopathogenic fungi over two species of sugarcane borer *Diatraea* spp. (Lepidoptera: Pyralidae)** – Gloria P. Barrera¹, Emiliano Barreto², Paula Sotelo¹, Paola Cuartas¹, Laura Villamizar¹, ¹Corporación Colombiana de Investigación Agropecuaria, Corpoica, Mosquera, Colombia; ²Centro de Bioinformática, Instituto de

- Biología, Universidad Nacional de Colombia, Bogotá, Colombia.
- FU-20** **Entomopathogenic fungi for the control of *Thaumastocoris peregrinus* Carpintero and Dellappé (Heteroptera: Thaumastocoridae) – *Sofía Simeto*¹, Ana B. Corallo², Sandra Lupo³, Lina Bettucci³, Demian Gómez¹, Paula González¹, Gonzalo Martínez¹, Eduardo Abreo², Federico Rivas², Nora Altier²**, ¹Instituto Nacional de Investigación Agropecuaria, Programa de Producción Forestal, E.E. del Norte, Tacuarembó; ²Instituto Nacional de Investigación Agropecuaria, Programa de Sustentabilidad Ambiental, Sección Bioinsumos, E.E. Wilson Ferreira Aldunate, Las Brujas, Canelones; ³Facultad de Ciencias/Ingeniería, Laboratorio de Micología, Universidad de la República, Montevideo.
- FU-21** **Effectiveness of *Metarhizium anisopliae* and *Beauveria bassiana* against eggs of tomato leafminer, *Tuta absoluta* Meyrick, 1917 (Gelichiidae: Lepidoptera) – *Laila A. M. Al-Shuraym*¹, Nagdy F. Abdel-Baky²**, ¹Department of Biology, College of Arts and Sciences in Buraydah, Qassim University, Saudi Arabia; ²Department of Plant Production & Protection, College of Agriculture and Veterinary Medicine Qassim University, P.O. Box: 6622, Buraydah 51452, Saudi Arabia.
- FU-22** ***Metarhizium* F52 microsclerotia applied in hydromulch to control Asian longhorn beetle – *Tarryn Anne Goble*¹, *Ann Hajek*², *Mark Jackson*², *Sana Gardescu*¹**, ¹Department of Entomology, Cornell University, Ithaca, NY, 14853-2601; ²USDA-ARS-NCAUR, Crop Bioprotection Research Unit, 1815 N University Street, Peoria, IL, 61604, USA.
- FU-23-STU** **Control of ticks (*Ixodes ricinus*) in sheep pastures in Norway with the fungal biocontrol agent BIPESCO 5 (*Metarhizium brunneum*) – *Natasha Iwanicki*¹, *Lise Grøva*², *Hermann Strasser*³, *Annette Folkedal Schjøll*², *Maria Björkman*⁴, *Karin Westrum*², *Jürg Enkerli*⁴, *Nicolai V. Meyling*⁵, *Ingeborg Kligen*²**, ¹Department of Entomology and Acarology, ESALQ, University of São Paulo, Brazil; ²Norwegian Institute for Agricultural and Environmental Research (Bioforsk), Norway; ³Institute of Microbiology, Leopold-Franzens University of Innsbruck, Austria; ⁴Swiss Federal Research Station for Agroecology and Agriculture, Switzerland; ⁵Department of Ecology, Faculty of Life Sciences, University of Copenhagen.
- FU-24-STU** **Development of *Aphidius colemani* incapable of oviposition and the evaluation of control efficacy against cotton aphid by combined use of entomopathogenic fungus and parasitoid wasp – *Fuka Oikawa*¹, *Minehiro Ishii*^{2,3}, *Masanori Koike*¹, *Daigo Aiuchi*⁴**, ¹Department of Agro-environmental Science, Obihiro University of Agriculture and Veterinary Medicine, Japan; ²The United Graduate School of Agricultural Sciences, Iwate University, Japan; ³Research Fellowship for Young Scientists, Japan Society for the Promotion of Science; ⁴Research Center for Global Agro-medicine, Obihiro University of Agriculture and Veterinary Medicine, Japan.
- FU-25** **Latent infection of wireworms and its relation to ambient *Metarhizium* levels in soil – *Todd Kabaluk*, *Erica Li-Leger***, Agriculture and Agri-Food Canada, Agassiz, BC, Canada.
- FU-26** **Smell the danger! Odor-perception of fungal infection risk in a below-ground parasitoid – *Belén Cotes*¹, *Linda-Marie Rännbäck*¹, *Maria Björkman*², *Hans R. Norli*², *Nicolai V. Meyling*³, *Birgitta Rämert*¹, *Peter Anderson*¹**, ¹Department of Plant Protection Biology, Division of Integrated Plant Protection, Swedish University of Agricultural Sciences, Växtskyddsvägen 3, P.O. Box 102, SE-230 53 Alnarp, Sweden; ²Bioforsk. Plant Health and Plant Protection Division, Høgskoleveien 7, 1430, Ås, Norway; ³Department of Plant and Environmental Sciences, University of Copenhagen, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark.
- FU-27-STU** **Maize endophytes offer potential protection against insects and disease – *Jenny J. Brookes*, *Travis R. Glare*, *Michael Rostás***, Bio Protection Research Centre, Lincoln University, New Zealand.
- FU-28** **Physiochemical characteristics of entomopathogenic fungi-derived antifungal substances – *Tae Young Shin*, *Sung Min Bae*, *Won Seok Gwak*, *Seung Hee Lee*, *Yong Oh Ahn*, *Soo Dong Woo***, Department of Agricultural Biology, College of Agriculture, Life & Environment Science, Chungbuk National University, Cheongju 361-763, Korea.
- FU-29-STU** **Entomopathogenic fungi “infecting” young lives – *Alap Sahoo*¹, *Sumanth S. R. Dara*¹, *Suchitra S. Dara*², *Surendra K. Dara*³**, ¹Massachusetts Institute of Technology, Cambridge, MA, USA; ²Warren Junior High School, Bakersfield, CA, USA; ³University of California Cooperative Extension, San Luis Obispo, CA, USA.

Microbial Control

- MC-1** **Next generation biopesticides for New Zealand’s key insect pests and plant diseases – *Maureen O’Callaghan*¹, *Travis Glare*², *Mark Hurst*¹, *Sean Marshall*¹, *Tracey Nelson*¹, *Michael Wilson*³, *Sarah Mansfield*¹, *Sue Zydenbos*¹**, ¹Innovative Farm systems, AgResearch, Lincoln Research Centre, New Zealand; ²Bio-Protection Research Centre, Lincoln University New Zealand; ³Innovative Farm systems, AgResearch, Ruakura New Zealand.
- MC-2** **Appearance of pathogens within outbreak populations of native porina caterpillar (*Wiseana* spp.) populations in New Zealand – *Sean D. G. Marshall*¹, *Richard J. Townsend*¹, *Colin M. Ferguson*², *Sarah Mansfield*¹**, ¹Innovative Farming Systems, AgResearch, Lincoln Research Centre, Christchurch, New Zealand; ²Innovative Farming Systems, AgResearch, Invermay Agricultural Centre, Mosgiel, New Zealand.
- MC-3** **Pathogens and nematodes of bark beetles (Coleoptera: Scolytidae) in Georgia coniferous forest – *Archil Supatasvili*, *Medea Burjanadze***, Department of Forest Protection, Vasil Gulisashvili Forest Institute, Agricultural University of Georgia, Tbilisi, Georgia.
- MC-4-STU** **PhopGV for control of *Tuta absoluta* in tomato and *Phthorimaea operculella* and *Tecia solanivora* in potato – *Andreas Larem*, *Eva Fritsch*, *Karin Undorf-Spahn*, *Johannes A. Jehle*, *Julius Kühn-Institut (JKI)***, Federal Research Centre for Cultivated Plants, Institute for Biological Control, Heinrichstr. 243, 64287 Darmstadt, Germany.

- MC-5-STU** **Sublethal effects of Cry1Ac on immune responses and baculovirus infection in *Spodoptera frugiperda*** – Lucas N. Wisch^{1,2,4}, Jenny S. Cory², Daniel R. Sosa-Gómez², ¹Pos-Graduation Program, State University of Ponta Grossa, Ponta Grossa, Paraná, Brazil; ²Dept. of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada; ³Entomology Research, Embrapa Soybean, Londrina, Paraná, Brazil.
- MC-6-STU** **Virulence of a newly isolated *Bacillus thuringiensis* strain against lepidopteran larvae** – Anna I. Moldovan^{1,2}, Natalia Munteanu Molotievskiy², Svetlana G. Bacal², Ion K. Toderas², ¹Faculty of Biology and Soil Science, Moldova State University, Chisinau, Republic of Moldova; ²Institute of Zoology, Academy of Sciences of Moldova, Chisinau, Republic of Moldova.
- MC-7** **Selection and characterization of formulation prototypes based of *Beauveria bassiana* for biological control of *Ceratomyxa tingomariana*** – Erika Grijalba¹, Lorena Garcia¹, Carlos Espinel¹, Judith Guevara, Hugo Fernando Rivera¹, ¹Biological Control Laboratory; ²Entomological Laboratory, Colombian Corporation for Agriculture Research, CORPOICA, Colombia.
- MC-8** **Sublethal effects of Cry1Ac *Bacillus thuringiensis* protein on *Plutella xylostella* (Lepidoptera: Plutellidae) Brazilian population: Life table study** – Caroline P. De Bortoli¹, Ricardo A. Polanczyk¹, Neil Crickmore², Sergio A. De Bortoli¹, Alessandra M. Vacari¹, ¹Department of Plant Protection, Sao Paulo State University, Jaboticabal, Sao Paulo, Brazil; ²Department of Biochemistry, School of Life Sciences, University of Sussex, Falmer, Brighton, United Kingdom.
- MC-9** **Interaction between predator *Podisus nigrispinus* (Dallas) and the entomopathogenic bacterium *Bacillus thuringiensis* Berliner** – Vanessa F. P. Carvalho, Sergio Antonio De Bortoli, Alessandra M. Vacari, Department of Plant Protection, Sao Paulo State University, Jaboticabal, Sao Paulo, Brazil.
- MC-10** **Reproduction and population parameters of predator *Podisus nigrispinus* (Dallas) providing *Bacillus thuringiensis* Berliner suspension as water source over generations** – Vanessa F. P. Carvalho, Sergio Antonio De Bertoli, Alessandra M. Vacari¹, Department of Plant Protection, Sao Paulo State University, Jaboticabal, Sao Paulo, Brazil.
- MC-11-STU** **Expression of recombinant ABCC2 from *Pectinophora gossypiella* and their influences on the cytotoxicity of activated Cry1Ac to Hi5 cells** – Yutao Xiao, The State Key Laboratory for Biology of Plant Disease and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, West Yuanmingyuan Road, Beijing, 100193, China.
- MC-12-STU** **Evaluation of commercial formulations of entomopathogenic fungi for managing the ambrosia beetles *Xylosandrus crassiusculus* and *Xyleborus volvulus* (Coleoptera: Curculionidae), vectors of the laurel wilt pathogen affecting avocado production in Florida** – Pasco B. Aveny¹, Verónica F. Bojorque^{1,2}, José M. Pérez-Martínez², Armando Monterroso³, Ronald D. Cave¹, J. Daniel Carrillo⁴, ¹University of Florida, IFAS, Indian River Research and Education Center, Fort Pierce, FL, USA; ²PanAmerican School of Agricultural, El Zamorano, Honduras; ³Brooks Tropicals, LLC, Homestead, FL, USA; ⁴University of Florida, IFAS, Tropical Research and Education Center, Homestead, FL, USA.
- MC-13** **Efficacy of *Metarhizium anisopliae* ICIPE 7 for the control of cattle tick** – Paulin Nana^{1,2}, Sunday Ekese¹, Mercy Mumbi³, Nguya K. Maniania¹, ¹International Centre of Insect Physiology and Ecology, Nairobi, Kenya; ²Faculty of Agriculture and Agricultural Sciences, University of Dschang, Cameroon; ³Department of Veterinary Pathology, Microbiology and Parasitology, University of Nairobi, Kenya.
- MC-14** **Morphological and physiognomic study of seven isolates of an entomopathogenic fungus** – Cavallo E. C., Marfetán J. A., Folgarait P. J., Laboratorio de Hormigas, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Bernal, Buenos Aires, Argentina.
- MC-15** **Delivery of entomopathogenic fungi as a seed coating for control of soil-dwelling insect pests** – Federico Rivas^{1,2,3}, Trevor Jackson², John Hampton¹, Per Wessman², Jayanthi Swaminathan², Michael Rostas¹, Travis R. Glare¹, ¹Bio-Protection Research Centre, Lincoln University, Lincoln, New Zealand; ²AgResearch, Lincoln Research Centre, Lincoln, New Zealand; ³National Institute for Agricultural Research, Las Brujas, Uruguay.
- MC-16** **Fat pellet strategy applied to the boll weevil (*Anthonomus grandis*) under laboratory conditions** – Marcos Faria, Lorena K. B. Bravo, Bruna M. Kraus, Rogério B. Lopes, EMBRAPA Genetic Resources and Biotechnology, Brasília, DF, Brazil.
- MC-17-STU** **Compatibility of fungicides with *Beauveria bassiana*** – Sumanth S. R. Dara¹, Suchitra S. Dara², Alap Sahoo¹, Haripriya Bellam¹, Surendra K. Dara³, ¹Stockdale High School, Bakersfield, CA, USA; ²Warren Junior High School, Bakersfield, CA, USA; ³University of California Cooperative Extension, San Luis Obispo, CA, USA.
- MC-18** **Characterization of *Tolypodium cylindrosporium* (Hypocreales: Ophiocordycipitaceae) and its effectiveness in infecting *Aedes aegypti* eggs (Diptera: Culicidae)** – Lina B. Flor-Weiler¹, Alejandro P. Rooney¹, Robert W. Behle¹, Daniel A. Strickman², ¹Crop Bioprotection Unit, Agricultural Research Service, U.S. Department of Agriculture, Peoria, IL 61604; ²(Retired), Office of National Programs, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD 20705, USA.
- MC-19** **Stress-induced changes in the dopamine levels of haemolymph of cabbage armyworm *Mamestra brassicae* and Colorado potato beetle *Leptinotarsa decemlineata*** – Chertkova E. A., Yaroslavtseva O. N., Dubovskiy I. M., Glupov V. V., Institute of Systematics and Ecology of Animals, Russian Academy of Sciences, Siberian Branch, Frunze str. 11, Novosibirsk 630091 Russia.
- MC-20** **Biosynthesis of silver nanoparticles using the fungus *Trichoderma viride* for the toxicity on *Aedes aegypti* mosquito and their antibacterial activity** – Siva Kamalakannan, Chandrakasan Gobinath, Kadarkarai Murugan, Unit of Medical Entomology, Department of Zoology, Bharathiar University, Coimbatore-641046, India.

Microsporidia

- MI-1** **Effects of the microsporidian pathogen, *Nosema adaliae* on the seven-spotted lady beetle, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae)** – *Entisar Elkabir, Susan Björnson*, Department of Biology, Saint Mary's University, 923 Robie Street, Halifax, NS, B3H 3C3, Canada.
- MI-2** **Online weather-based risk forecast model for *Nosema* spp. infections in German honey bee apiaries and projected risk under climate change** – *Anto Raja Dominic¹, Sebastian Gisder², Elke Genersch², Andreas Linde¹*, Hochschule für nachhaltige Entwicklung Eberswalde, Dept. of Forest and Environment, Alfred-Möller-Str. 1, 16225, Eberswalde, Germany; ¹Länderinstitut für Bienenkunde Hohen Neuendorf e.V., Friedrich-Engels-Str. 32, 16540, Hohen Neuendorf, Germany.
- MI-3** **rRNA organization in a new light: A microsporidium infecting mountain pine beetle, *Dendroctonus ponderosae* (Coleoptera: Scolytidae)** – *George Kyei-Poku, Debbie Gauthier*, Natural Resources Canada, Canadian Forest Service, Great Lakes Forestry Centre, 1219 Queen Street East, Sault Ste. Marie, Ontario, Canada.
- MI-4** **Light and electron microscopic observations of *Anncalia* sp. (Microsporidia: Tubulinosematidae) from *Dikerogammarus villosus* (Amphipoda: Gammaridae)** – *Yuri S. Tokarev¹, Yuliya Y. Sokolova^{2,3}, Irma V. Issi¹*, ¹Institute for Plant Protection; ²Institute of Cytology, Russian Academy of Sciences, St. Petersburg, Russia; ³School of Veterinary Medicine, Louisiana State University, Baton Rouge LA, USA.

Nematodes

- NE-1** **Improved survival time of infective juveniles of *Steinernema glaseri* collected on Paris plaster** – *C. I. Cortés-Martínez¹, J. Ruiz-Vega¹, E. E. Lewis²*, ¹Instituto Politécnico Nacional, CIIIDIR-Oaxaca, Oaxaca, 71230. México; ²Department of Nematology, University of California, Davis, Davis, CA 95616, USA.
- NE-2** **Ecological characterization of *Steinernema siamkayai* (Rhabditida: Steinernematidae), a warm-adapted entomopathogenic nematode isolate from India** – *Ramalingam Karthik Raja^{1,2}, Aishwarya Dilipkumar¹, Sivaperumal Sivaramakrishnan³, Selcuk Hazir², Pachiappan Perumal¹*, ¹Department of Biotechnology, Periyar University, Salem, Tamil Nadu, India; ²Faculty of Arts and Science, Department of Biology, Adnan Menderes University, 09010 Aydin, Turkey; ³Department of Biotechnology and Genetic Engineering, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India.
- NE-3** **Evaluation of entomopathogenic nematodes *Steinernema feltiae* and *Heterorhabditis bacteriophora* against melon aphid (*Aphis gossypii* Glow, Hemiptera Aphididae)** – *Nona Mikaia*, Department of Natural Sciences and Health Care, Sokhumi State University, 9, Anna Politkovskaya Str. Tbilisi 0186, Georgia.
- NE-4-STU** **A new way for number regulation of South American tomato moth, *Tuta absoluta* in Georgia** – *Mariam Chubinishvili, Tsisia Chkhubanishvili*,

Manana Kakhadze, Iatamze Malania, Rusudan Skhirtladze, Irine Rizhamadze, Biological Control Laboratory, Kanchaveli Institute of Plant Protection, Agricultural University of Georgia.

- NE-5** **Roots exudates favor friends and hinder foes in the rhizospheric nematode community** – *L. Hiltbold¹, G. Jaffuel², T.C.J Turlings²*, ¹Hawkesbury Institute for the Environment, University of Western Sydney, Penrith, NSW, Australia; ²FARCE lab, University of Neuchâtel, Neuchâtel, Switzerland.
- NE-6-STU** **Host searching behavior of entomopathogenic nematode from the subarctic zone in Japan under low temperature** – *Yohsuke Maquchi¹, Toyoji Yoshiga², Daigo Aiuchi³, Masanori Koike¹*, ¹Department of Agro-environmental Science, Obihiro University of Agriculture & Veterinary Medicine, Obihiro, Japan; ²Laboratory of Nematology, Department of Applied Biological Sciences, Faculty of Agriculture, Saga University; ³Research Center for Global Agro-medicine, Obihiro University of Agriculture and Veterinary Medicine, Japan.
- NE-7** **Genomic determinants of the entomopathogenic lifestyle from independently arising nematodes** – *Hillel Schwartz¹, Adler Dillman², Paul Sternberg¹*, ¹HHMI and Division of Biology and Biological Engineering, California Institute of Technology, Pasadena CA 91125, USA; ²Department of Nematology, University of California Riverside, CA 92521, USA.

Viruses

- VI-1-STU** **The impact of virus diversity on the evolution of pest resistance** – *Leon Yu Zheng Li¹, Jenny S. Cory¹*, ¹Department of Biological Sciences, Simon Fraser University, Burnaby, BC, Canada.
- VI-2** **Constitutive and herbivore-induced defenses of soybean inhibits baculoviral disease in the fall armyworm, *Spodoptera frugiperda*** – *Ikkei Shikano, Kelli Hoover*, Department of Entomology, Pennsylvania State University, University Park, PA, USA.
- VI-3-STU** **Three baculoviruses infecting white satin moth (*Lesanpv*), douglas-fir tussock moth (*OpMNpv*) and pale tussock moth (*DapuNPV*) cluster together on the phylogenetic tree** – *Martyna Krejmer¹, Lukasz Rabalski¹, Iwona Skrzeczek², Jadwiga Ziemnicka³, Boguslaw Szewczyk¹*, ¹Intercollegiate Faculty of Biotechnology University of Gdansk and Medical University of Gdansk, Department of Recombinant Vaccines, Gdansk, Poland; ²Forest Research Institute, Department of Forest Protection, Sekocin Stary, Poland; ³Institute of Plant Protection, Department of Biological Control and Quarantine, Poznan, Poland.
- VI-4** **Screening field collected Lepidoptera larvae for new virus isolates** – *Michelle T. Franklin¹, Amy Huang¹, Yan Han¹, Matilda Tabert¹, Martin Erlandson², Stefan Richard³, Deborah Henderson¹*, ¹Institute for Sustainable Horticulture, Kwantlen Polytechnic University, Langley, BC, Canada; ²Saskatoon Research Centre, Agriculture and Agri-Food Canada, Saskatoon, SK, Canada; ³Sylvar Technologies Inc., Fredericton, New Brunswick, Canada.
- VI-5-STU** ***Culex pipiens* associated Tunis virus: A new mosaic virus in common house mosquitos** – *Diane Bigot¹*,

- Elisabeth A. Herniou¹, Marion Ballenghien², Mylène Weill², Célestine Atyame², Nicolas Galtier², Philippe Gayral¹, ¹Institut de Recherche sur la Biologie de UMR 7261, CNRS, Université François-I-Insecte, Rabelais, 37200 Tours, France; ²Institut des Sciences de l'Evolution de Montpellier, UMR 5554, CNRS, Université Montpellier 2, Place E. Bataillon, 34095 Montpellier, France.
- VI-6 Newly sequenced full genome of Nun moth (*Lymantria moanacha*) baculovirus show its high similarity to gypsy moth (*Lymantria dispar*) baculovirus** – *Lukasz Rabalski*¹, *Martyna Krejmer*¹, *Iwona Skrzecz*², *Bartosz Wasag*³, *Boguslaw Szewczyk*¹, ¹Intercollegiate Faculty of Biotechnology University of Gdansk and Medical University of Gdansk, Department of Recombinant Vaccines, 80-822 Gdansk, Kladki Str. 24, Poland; ²Forest Research Institute, Department of Forest Protection, 05-090 Raszyn, Braci Lesnej Str. 3, Sekocin Stary, Poland; ³Medical University of Gdansk, Department of Biology and Genetics, 80-211 Gdansk, Debinki Str. 1, Poland.
- VI-7 Comparison among betabaculovirus isolates from Gelechiidae insect family** – *Gloria P. Barrera*¹, *Juliana A. Gómez*², *Mariano N. Belaich*², *Pablo D. Ghiringhelli*², *Laura Villamizar*¹, ¹Corporación Colombiana de Investigación Agropecuaria, Corpoica, Bogotá, Colombia; ²Laboratorio de Ingeniería Genética y Biología celular y Molecular, Área Virosis de Insectos (LIGBCM-AVI), Dto. De Ciencia y Tecnología, Universidad Nacional de Quilmes, Provincia de Buenos Aires, Argentina.
- VI-8 Genome sequence analysis of a two alphabaculoviruses and a betabaculovirus from armyworms of genus *Mythimna*** – *Robert L. Harrison*¹, *David A. Theilmann*², *Martin A. Erlandson*³, ¹USDA Agricultural Research Service, Beltsville, MD, USA; ²Agriculture and Agri-Food Canada, Summerland, BC, Canada; ³Agriculture and Agri-Food Canada, Saskatoon, SK, Canada.
- VI-9 Genome sequence of *Trichoplusia ni* granulovirus (TnGV), a novel sequenced betabaculovirus infecting the cabbage looper** – *Ma. de los Angeles Bivián-Hernández*¹, *Juventino López-Tlajomulco*², *Jorge E. Ibarra*², *Ma. Cristina Del Rincón-Castro*¹, ¹Food Department and Graduate Program in Biosciences, University of Guanajuato, Irapuato, GTO, México; ²Department of Biotechnology and Biochemistry, CINVESTAV-Irapuato, Irapuato, GTO, México.
- VI-10-STU The gene *pp31* of *Cydia pomonella* granulovirus is essential for the production of budded viruses and the establishment of a systemic infection in codling moth** – *Jacqueline A. Frank*, *Manuela Gebhardt*, *Diana Schneider*, *Jörg T. Wennmann*, *Johannes A. Jehle*, Institute for Biological Control, Julius Kühn Institute (JKI), Federal Research Center on Cultivated Plants, Heinrichstraße 243, 64287 Darmstadt, Germany.
- VI-11-STU Membrane binding and fusion play no role in cross-resistance against a granulovirus in a strain of *Adoxophyes honmai* selected for resistance to a nucleopolyhedrovirus** – *Kento Iwata*, *Maki N. Inoue*, *Yasuhisa Kunimi*, *Madoka Nakai*, Tokyo University of Agriculture and Technology, Saiwai, Fuchu, Tokyo 183-8509, Japan.
- VI-12 Enhancers and chitinases: Analysis of a granulovirus of *Spodoptera frugiperda*** – *Paola E. Cuartas*¹, *Emiliano Barreto*², *Gloria P. Barrera*¹, *Laura F. Villamizar*¹, ¹Corporación Colombiana de Investigación Agropecuaria, Corpoica, Bogotá, Colombia; ²Centro de Bioinformática, Instituto de Biotecnología-IBUN, Universidad Nacional de Colombia, Bogotá, Colombia.
- VI-13 Heterologous recombination between Baculoviruses: Horizontal transfer genes analysis** – *Gloria P. Barrera*¹, *Mariano N. Belaich*², *Paola Cuartas*¹, *Laura Villamizar*¹, *Pablo D. Ghiringhelli*², ¹Corporación Colombiana de Investigación Agropecuaria, Corpoica, Bogotá, Colombia; ²Laboratorio de Ingeniería Genética y Biología celular y Molecular, Área Virosis de Insectos (LIGBCM-AVI), Dto. De Ciencia y Tecnología, Universidad Nacional de Quilmes, Provincia de Buenos Aires, Argentina.
- VI-14 Establishment of a winter moth, *Operophtera brumata*, cell line permissive for OpbrNPV replication** – *John P. Burand*¹, *Robert L. Harrison*², *Matthew Boucher*¹, ¹Department of Microbiology, University of Massachusetts Amherst, MA, USA; ²USDA Agricultural Research Service, Beltsville, MD, USA.
- VI-15 AcMNPV infection process in *Trichoplusia ni* midgut** – *Muhammad Afzal Javed*¹, *Stephanie Harris*¹, *David A. Theilmann*², *Martin A. Erlandson*¹, *Dwayne D. Hegedus*¹, ¹Saskatoon Research Centre, Agriculture and Agri-Food Canada, Saskatoon, SK, S7N 0X2 Canada; ²Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Summerland, BC, VOH 1Z0 Canada.
- VI-16-STU The effects of heterologous *p10* expression in *Autographa californica* multicapsid nucleopolyhedrovirus replication in insect cells** – *Leo Graves*¹, *Sarah L. Irons*¹, *Robert D. Possee*^{1,2}, *Linda A. King*¹, ¹Department of Biological and Medical Sciences, Oxford Brookes University, Oxford UK; ²Oxford Expression Technologies Ltd, Oxford, UK.
- VI-17-STU Multiple amino acid residues of *Autographa californica* MNPV P143 are responsible for ribosomal RNA degradation in *Bombyx mori* cells** – *Rina Hamajima*, *Michihiro Kobayashi*, *Motoko Ikeda*, Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya 464-8601, Japan.
- VI-18 *Autographa californica* multiple nucleopolyhedrovirus ORF11 and ORF78 are essential for budded virus production, occlusion-derived virus envelopment, and occlusion body formation** – *Xue Ying Tao*^{1,2}, *Woo Jin Kim*³, *Jae Young Choi*⁴, *Seok Hee Lee*³, *Jong Hoon Kim*³, *Pang Ying*³, *Ha Kyu Baik*³, *Yeon Ho Je*³, ¹State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang, China; ²Jiangxi-OAI Joint Research Institute, Nanchang University, Nanchang, China; ³College of Agriculture and Life Science, Seoul National University, Seoul, Republic of Korea; ⁴Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul, Republic of Korea.
- VI-19-STU Effects of lacking non-essential genes of BmNPV** – *Hitomi Taka*¹, *Chikako Ono*², *Masanao Sato*³, *Shin-ichiro Asano*¹, *Hisanori Bando*¹, ¹Graduate School of Agriculture, Hokkaido University, Sapporo, Japan; ²Research Institute for Microbial Diseases, Osaka University, Suita, Japan; ³Institute for Advanced Biosciences, Keio University, Tsuruoka, Japan.

- VI-20-STU** **Relevance of BmSynDecan-1 for BmNPV proliferation** – *Momoka Uda¹, Masanao Sato², Shin-ichiro Asano¹, Hisanori Bando¹*, ¹Graduate School of Agriculture, Hokkaido University, Sapporo, Japan; ²Institute for Advanced Biosciences Keio University, Yamagata, Japan.
- VI-21-STU** **Applications of DnaB mini-intein to baculovirus expression system** – *Won Seok Gwak, Sung Min Bae, Tae Young Shin, Seung Hee Lee, Yong Oh Ahn, Soo Dong Woo*, Department of Agricultural Biology, College of Agriculture, Life & Environment Science, Chungbuk National University, Cheongju 361-763, Korea.
- VI-22-STU** **Production of porcine parvovirus virus-like particles using baculovirus in the silkworm larvae** – *Seung Hee Lee, Sung Min Bae, Tae Young Shin, Won Seok Gwak, Yong Oh Ahn, Soo Dong Woo*, Department of Agricultural Biology, College of Agriculture, Life & Environment Science, Chungbuk National University, Cheongju 361-763, Korea.
- VI-23** **Transcriptional analysis of the putative glycosyl transferase gene (amv248) of *Amsacta moorei* entomopoxvirus** – *Cihan Inan¹, Hacer Muratoglu², Basil Arif³, Zihni Demirbağ¹*, ¹Department of Biology, Karadeniz Technical University, Trabzon, Turkey; ²Department of Molecular Biology and Genetics, Faculty of Sciences, Karadeniz Technical University, Trabzon, Turkey; ³Laboratory for Molecular Virology, Great Lakes Forestry Centre, Sault Ste. Marie, Ontario, Canada.
- VI-24-STU** **CrV1 mimics host α -tubulin to sequester GAPDH, which plays a crucial role in cytoskeleton rearrangement** – *Sunil Kumar, Yonggyun Kim*, Department of Bioresource Sciences, Andong National University, Andong 760-749, Republic of Korea.
- VI-25** **The use of zinc-based fixatives for high-fidelity histomorphology and molecular histochemical techniques on arthropod tissue** – *Damien M. Laudier*, Laudier Histology, New York City, NY, USA.

SIP Division Business Meetings Wednesday 20:00 – 22:00

Fungi	2311
Microsporidia (+Workshop)	2314
Viruses (+Workshop)	2301
Bacteria	2306
Nematode	2309

Workshop Wednesday 20:00 – 22:00
Microsporidia 2314

War and Peace: The Comparative Impact of Morphology- and Sequence-based Phylogenies on Practical Taxonomy and Evolutionary History of Microsporidia

Organizer: Yuliya Sokolova

Workshop Wednesday 20:00 – 22:00
Viruses 2301

Definition of Virus Species Concept

Organizers: Holly Popham and Robert Harrison

THURSDAY – August 13

7:30 – 12:00 Information & Registration Outside Great Hall

Symposium Thursday 8:00 – 10:00
Nematode 2301

Recent Advances in Entomopathogenic Nematode Infection Behaviour: Inside and Outside

Moderators: David Shapiro-Ilan and Ed Lewis

- 8:00 **125 Advances in entomopathogenic nematode dispersal and host-finding behavior** – *David Shapiro-Ilan¹, Edwin E. Lewis²*, ¹USDA-ARS, SEFTNRL, Byron, GA, USA; ²Department of Entomology and Nematology, University of California, Davis, USA.
- 8:30 **126 Sex, age and following the leader drive infection dynamics of entomopathogenic nematodes** – *Ed Lewis¹, David Shapiro-Ilan², Yohandra Gonzales¹, Danica Maxwell¹*, ¹Department of Entomology and Nematology, University of California – Davis, Davis, CA, USA; ²USDA-ARS, SEFTNRL, Byron, GA, USA.
- 9:00 **127 Impact of infection behaviour on lethal male fighting in *Steinernema*** – *Apostolos Kapranas, Abigail M. D. Maher, Annemie Zenner, Kate O'Callaghan, Christine T. Griffin*, Biology Department, Maynooth University, Maynooth, County Kildare, Ireland.
- 9:30 **128 The stability of virulence in insect parasitic nematodes is determined by social interactions** – *Ben Raymond¹, David Shapiro-Ilan²*, ¹Imperial College London, Silwood Park campus, Ascot, Berks, SL5 7PY, UK; ²USDA-ARS, South Eastern Fruit and Nut Research, Byron, USA.

Contributed Papers Thursday 8:00 – 10:00
2306/9

Fungi 2

Moderators: Stefan Jaronski and Ann Hajek

- 8:00 **129-STU Transcriptomic and physiological responses of *Arabidopsis thaliana* to endophytic *Beauveria bassiana*** – *Maya Raad, Travis Glare, Michael Rostás*, Bio-Protection Research Centre, PO Box 85084, Lincoln University, Lincoln 7647, New Zealand.
- 8:15 **130 Non-entomopathogenic role of entomopathogenic fungi in strawberry production** – *Surendra K. Dara*, University of California Cooperative Extension, San Luis Obispo, CA, USA.
- 8:30 **131-STU Phenotypic diversity among isolates of the entomopathogenic endophyte *Beauveria bassiana* affects plant-host interactions** – *Aimee McKinnon¹, Travis R. Glare¹, Hayley Ridgway², Andrew Holyoake¹, Artemio Mendoza Mendoza¹*, ¹Bio-Protection Research Centre, Lincoln University, Christchurch, New Zealand; ²Faculty of Agriculture and Life Sciences, Lincoln University, Christchurch, New Zealand.
- 8:45 **132 Light affect sporulation patterns of the mitogenic pathogenic fungus *Neozygites floridana*** – *Ingeborg Klinagen¹, Maren Pindsl Holthe^{1,2}, Aruppillai Suthaparan², Karin Westrum¹, Torfinn Torp¹*, ¹Norwegian Institute for

- Agricultural and Environmental Research (Bioforsk), Norway; ²Norwegian University of Life Sciences, Department of Plant Sciences, Norway.
- 9:00 **133 Environmental safety of Canadian *Metarhizium* S54 for the northern crayfish, *Orconectes virilis*, and phantom midge larva, *Chaoborus americanus* – Dan Johnson¹, Larry Kawchuk², Stefan Jaronksi³, Craig Wiebe¹, Zhe Zhang², ¹Water and Environmental Science Building, University of Lethbridge, Lethbridge, Alberta, Canada T1K 3M4; ²Research Centre, Agriculture and Agri-Food Canada, Lethbridge, Alberta, Canada T1J 4B1; ³Pest Management Research Unit, Northern Plains Agricultural Research Laboratory, USDA Agricultural Research Service, Sidney, Montana, USA.**
- 9:15 **134 Temporal density dependence of *Entomophaga maimaiga* – Ann E. Hajek**, Department of Entomology, Cornell University, Ithaca, NY, USA.
- 9:30 **135 Importance of mechano-signal for fungus removal in *Drosophila* grooming – Aya Yanaqawa¹, Toshimitsu Hata¹, Tsuyoshi Yoshimura¹, Frederic Marion-Poll^{2,3}, ¹Kyoto University, Uji, Japan; ²CNRS, Laboratoire Evolution, Génomes, Comportement et Ecologie, Gif-sur-Yvette, France; ³AgroParisTech, Département Sciences de la Vie et Santé, Paris, France.**
- 9:45 **136-STU Older beetles are stronger than young: Influence of mating and age on susceptibility to a fungal pathogen – Joanna J. Fisher, Ann E. Hajek**, Department of Entomology, Cornell University, Ithaca NY, USA.

Contributed Papers Thursday 8:00 – 10:00
2311

Microbial Control 3

Moderator: Dietrich Stephan

- 8:00 **137-STU Toxicity of *Bacillus thuringiensis* culture filtrate to *Meloidogyne incognita* – Jiaheling Qi^{1,2}, Daigo Aiuchi², Shin-ichiro Asano³, Masanori Koike², ¹The United Graduate School of Agricultural Sciences, Iwate University, Iwate Japan; ²Department of Agro-environmental Science, Obihiro University of Agriculture & Veterinary Medicine, Obihiro, Japan; ³Department of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Sapporo, Japan.**
- 8:15 **138 *Yersinia entomohaga*: A biopesticidal bacterium active against a wide range of insect pests – Mark Hurst¹, Colin Ferguson², Sarah Mansfield¹, Richard Townsend¹, Sean Marshall, Sandra Jones¹, Jayanthi Swaminathan¹, David Wright¹, Michael Wilson³, Derick Wilson³, Maureen O'Callaghan¹, ¹Innovative Farm systems, AgResearch, Lincoln Research Centre, New Zealand; ²Innovative Farm systems, AgResearch Invermay Agricultural Centre, New Zealand; ³Innovative Farm systems, AgResearch, Ruakura New Zealand.**
- 8:30 **139 Toxicological and protein characterization of *Bacillus sphaericus* C3-41 strain from Karnataka – Basavaraj S. Kalmath¹, A. Prabhuraj², Katkar Gajanan³, R. S. Giraddi⁴, B. V. Patil⁵, ^{1,4}College of Agriculture Bheemaranagudi; ^{2,5}College of Agriculture Raichur; ³Department of Biochemistry, Mysore University.**
- 8:45 **140 A new invasive biotype of the coconut rhinoceros beetle (*Oryctes rhinoceros*) has escaped from biological control by *Oryctes rhinoceros* nudivirus – Sean D. G. Marshall¹, Maclean Vaqalo², Aubrey Moore³, Roland J.**

Quitugua³, Trevor A. Jackson¹, ¹Innovative Farming Systems, AgResearch, Lincoln Research Centre, Christchurch, New Zealand; ²Land Resources Division, Secretariat of the Pacific Community, Suva, Fiji Islands; ³College of Natural and Applied Sciences, University of Guam, GU, USA.

- 9:00 **141 Production of *Oryctes nudivirus* in DSIR-HA-1179 insect cell cultures in roller bottle systems – Gabriel Visnovsky¹, Charlotte Pushparajan¹, Juan D. Claus², ¹Department of Chemical and Process Engineering, University of Canterbury, New Zealand; ²Lab. Virología, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina.**
- 9:15 **142 Control of *Chrysodeixis includens* (Walker) and *Anticarsia gemmatalis* Hübner with *Bacillus thuringiensis*, chlorantraniliprole, and a mixture of PsiSNPV and AgMNPV – Daniel R. Sosa-Gómez**, Embrapa Soybean, Londrina, PR, Brazil.
- 9:30 **143 Fungal endophytes as first line of defense against the bean stem maggot *Ophiomyia phaseoli* (Tyron) on *Phaseolus vulgaris* (L.) – Beritah Mutune^{1,2}, Sunday Ekese¹, Saliou Niassy¹, Vivienne Matiru², Christine Bii², Nguya K. Maniania¹, ¹International Centre of Insect Physiology and Ecology (*icipe*), P. O. Box 30772-00100, Nairobi, Kenya; ²Jomo Kenyatta University of Agriculture and Technology (JKUAT), P.O. Box 62000-00200, Nairobi, Kenya.**
- 9:45 **144 Performance of three Indian isolates of *Beauveria bassiana* (Balsamo) Vuillemin and three commercial mycoinsecticides against three developmental stages of *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) – Mohammed I. Elbashir¹, P. Bishwajeet², K. Shankarganesh³, P. Sharma⁴, ¹Department of Bio pesticides and Biofertilizers- Environment and Natural Resources and Desertification Research Institute, P.O. Box 6096, Khartoum, Sudan; ^{2,3}Biological Control Laboratory Division of Entomology, Indian Agricultural Research Institute, New Delhi-110012; ⁴Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi-110012.**

10:00 – 10:30

BREAK

Thursday 10:30 – 12:30

Great Hall

Society for Invertebrate Pathology Annual Business Meeting

Presiding: Peter Krell

12:30 – 14:00

LUNCH (on your own)

Symposium Thursday 14:00 – 16:00
Microbial Control 2301

Synergies Enabling the Registration and Adoption of Biological Pest Controls – The Role of Governments, and Academic Programmes and Industry

Moderator: Tobias Laengle

- 14:00 **145 Facilitating the registration and adoption of biological pest controls in Canada – Tobias Laengle**, Pesticide Risk Reduction Program, AAFC, St. John's, Newfoundland & Labrador, Canada.

- 14:25 **146 The IR-4 biopesticide and organic support program** – Michael Braverman, *William Barney*, Interregional Research Project Number 4 (IR-4), Rutgers University, Princeton, NJ, USA.
- 14:50 **147 How does academia contribute to registration and adoption of biological control agents, a European perspective** – *Jørgen Eilenberg*, Department of Plant and Environmental Sciences, University of Copenhagen, Denmark.
- 15:15 **148 Perils and pitfalls of product development and commercialization: An industry perspective** – *Randy Martin*, Valent BioSciences Corporation, Libertyville, Illinois, USA.
- 15:40 Discussion

Contributed Papers Thursday 14:00 – 15:30
2306/9

Viruses 5

Moderators: Adly Abd-Alla and Umut Toprak

- 14:00 **149 Peritrophic matrix proteomics of the cotton leaf worm, *Spodoptera littoralis* (Lepidoptera: Noctuidae): Targets for pathogens infecting *per os*** – *Umut Toprak*¹, *Dwayne Hegedus*², *Esengul Ozdemir*¹, *Doug Baldwin*², *Cathy Coult*², *Diney Bekkaoui*², *Serife Bayram*¹, *M. Oktay Gürkan*¹, ¹Ankara University, Faculty of Agriculture, Dept. of Plant Protection, Ankara, Turkey; ²Agriculture and Agri-Food Canada, Saskatoon Research Centre, Saskatoon, SK, Canada.
- 14:15 **150 Analysis of occlusion derived virus infection in tissue culture using gene knock out viruses of the *per os* infectivity factors and GP64** – *David A. Theilmann*¹, *Leslie G. Willis*¹, *Michael Weis*¹, *Cam Donly*², *Dwayne D. Hegedus*², *Martin A. Erlandson*³, ¹Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Summerland, British Columbia, Canada V0H 1Z0; ²Southern Crop Protection and Food Research Agri-Food Canada, Saskatoon, SK, Canada.
- 14:30 **151 Inhibition of *Cotesia vanessae* development in *Trichoplusia ni* larvae infected with alphabaculoviruses from *Mamestra configurata*** – *Martin A. Erlandson*^{1,2}, *Stephanie Harris*¹, *Edyta Sieminska*², *Doug Baldwin*¹, *Dwayne D. Hegedus*¹, *David A. Theilmann*³, ¹Agriculture and Agri-Food Canada, Saskatoon Research Center, Saskatoon, SK, Canada; ²Department of Biology, University of Saskatchewan, Saskatoon, SK, Canada; ³Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, Summerland, BC, Canada.
- 14:45 **152 First successful elimination of an insect virus, *Glossina pallidipes* salivary gland hypertrophy virus, from insect factory: A model for managing insect viruses in insect factories for food and feed** – *Adly M. M. Abd-Alla*, Insect Pest Control Laboratory, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, Vienna, Austria.
- 15:00 **153 Impact of valacyclovir on the pathology of Hytrosavirus in *Musca domestica*** – *D. G. Boucias*¹, *C. Geden*², *A. M. M. Abd-Alla*³, ¹Entomology and Nematology Department, University of Florida, Gainesville, FL USA; ²Center for Medical, Agricultural and Veterinary USDA, ARS, Gainesville, FL 32608, USA; ³Insect Pest Control Laboratory, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Austria.
- 15:15 **154 Pathogen-host interaction of deformed wing virus (DWV) and the honey bee (*Apis mellifera*)** – *Sebastian Gisder*, *Elke Genersch*, Institute for Bee Research, Molecular Microbiology and Bee Pathology, Hohen Neuendorf, Germany.

Contributed Papers Thursday 14:00 – 16:00
2311

Bacteria 3

Moderators: Yulin Gao and Hyun-Woo Park

- 14:00 **156 Cyt1Aa-BinA chimera highly toxic to anopheline, aedine, and culicine larvae including those tolerant or resistant to *Lysinibacillus sphaericus*** – *Dennis K. Bideshi*^{1,2}, *Hyun-Woo Park*^{1,2}, *Robert H. Hice*¹, *Margaret C. Wirth*¹, *Brian A. Federici*^{1,3}, ¹Department of Entomology, University of California, Riverside, California, USA; ²Department of Natural and Mathematical Sciences, California Baptist University, Riverside, California, USA; ³Institute for Integrative Genome Biology, University of California, Riverside, California, USA.
- 14:15 **157 Effect of single versus multiple promoters and a high plasmid copy number on the synthesis and assembly of Cyt1Aa crystals in *Bacillus thuringiensis*** – *Hyun-Woo Park*¹, *Robert H. Hice*², *Brian A. Federici*², ¹Department of Entomology, University of California, Riverside, and Department of Natural and Mathematical Sciences, California Baptist University, Riverside, California, USA; ²Interdepartmental Graduate Programs in Microbiology and Cell, Molecular, and Developmental Biology, University of California, Riverside, Riverside, California, USA.
- 14:30 **158 The island of mosquitocidal toxins in *Bt* strain 2160-1 identified by whole genome sequencing and proteogenomic analysis** – *Jim X. J. Fang*^{1,2,3}, *Y. Zhou*², *P. T. Gong*², *Y. J. Wei*³, *Y. Zhang*³, ¹The HITAR Institute Canada Inc., Richmond, British Columbia, Canada; ²Hainan Institute of Tropical Agricultural Resources, Sanya, Hainan, China; ³Institute of Life Sciences, Jiyang College of Zhejiang A&F University, Zhuji, Zhejiang, China.
- 14:45 **159 Engineering of *Bacillus thuringiensis* Cry proteins to improve the activity against western corn rootworm** – *Ruth Cong*, *Hana Ali*, *Michi Izumi Wilcoxon*, *Yi Zheng*, *Jingtong Hou*, *Mark Nelson*¹, *Ericka Bermudez*, *Mark McDonald*, *Takashi Yamamoto*, Plant Protection, Ag Biotechnology, DuPont Pioneer, Hayward, CA and ¹Wilmington, DE, USA.
- 15:00 **160 Laboratory-selected western corn rootworm colony resistant to mCry3A** – *Jian-Zhou Zhao*, *Meghan Oneal*, *Nina Richtman*, *Analiza Alves*, *Stephen Thompson*, DuPont Pioneer, Johnston, IA, U.S.A.
- 15:15 **161 A new Cry1Ac toxin of *Bacillus thuringiensis* highly toxic to *Manduca sexta* and *Trichoplusia ni*** – *Yaritza Reinoso-Pozo*¹, *Cristina Del Rincón-Castro*², *Jorge E. Ibarra*¹, ¹Department of Biotechnology and Biochemistry, CINVESTAV-Irapuato, Gto. Mexico; ²Life Sciences Division, University of Guanajuato, Irapuato, Gto. Mexico.
- 15:30 **162 Rapid evolution and genetic basis of resistance to Cry1F in a lab-selected Asian corn borer and its cross-resistance to other Cry toxins** – *Yueqin Wang*, *Yidong Wang*, *Zhenying Wang*, *Kanqilai He*, State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protect, Chinese Academy of Agricultural Sciences, Beijing 100193, China.

15:45	163 Evaluation of <i>Bt</i> corn with stacked genes for resistance to the Asian corn borer – <i>Tiantao Zhang, Fan Jiang, Zhenying Wang, Kanglai He</i> , State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China.	16:15	Students Affairs Business Meeting	2311
		18:00	BANQUET	Great Hall

ABSTRACTS

2015

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STU Indicates **STUDENT** presentation

000 Indicates number of **ORAL** presentation

BA - 00 Indicates abstract number for **POSTER** presentation

MONDAY – August 10th

PLENARY SESSION Monday 10:30 – 12:30

**Insect Pathogens in Nature:
Ecology and Evolution**PLENARY SESSION Monday 10:30 **1****How sea stars get wasted: Evidence of a viral etiology and host response to sea star wasting disease***Colleen A. Burge, Team SSWD, Team EIMD*

The Institute of Marine and Environmental Technology, University of Maryland Baltimore County, Baltimore, MD, USA

Correspondence: colleenb@umbc.edu

A large-scale mortality event affecting 20 species of sea star (Asteroidea) on the Pacific Coast of North America began in June of 2013. First detected in Ochre sea stars (*Pisaster ochraceus*) along the outer coast of Washington State, the event has been observed in at least 250 subtidal and intertidal locations extending from Southeastern Alaska to San Diego, California. Citizen science has been a key approach to monitoring the spread of disease. *Pisaster*, *Pycnopodia* and *Evasterias* are the most affected genera and mortality approaches 100% in select species at some locations. Both *Pisaster* and *Pycnopodia* are considered keystone species, and changes to community composition within ecosystems are possible. The progression of clinical signs includes: 1) loss of body turgor (deflation) and weakness; 2) curling of rays; 3) foci of epidermal pallor and tissue loss; 4) sloughing of multiple rays and/or rupture of the body wall with evisceration; 5) death. Evidence from multiple approaches, including microscopic (histology and electron microscopy), genomic (metagenomics, community finger-printing, and transcriptomics), culture, and experimental (infection trials) led to a diagnosis of a viral etiology and a densovirus (*Parvoviridae*) as a candidate causative agent. *Pycnopodia helianthoides* coelomocyte response measured by a transcriptomic analysis indicates both an immune and nervous system response to exposure.

PLENARY SESSION Monday 11:00 **2****Symbiont-mediated defense against parasitic nematodes in *Drosophila****Steve J. Perlman*

Department of Biology, University of Victoria, Victoria, BC, Canada

Correspondence: stevep@uvic.ca

Terrestrial arthropods commonly harbour bacterial endosymbionts that are transmitted primarily from mothers to their offspring, often in the egg cytoplasm. These inherited symbionts, of which *Wolbachia* is the best known and most widespread, are major players in the ecology and evolution of their hosts. One recent exciting development in the study of inherited symbionts is the discovery that many protect their hosts against diverse natural enemies, including pathogenic fungi, parasitoid wasps, and RNA viruses. In this talk, I will discuss one of the best examples of defensive symbiosis operating in the wild, whereby a *Spiroplasma* bacterial symbiont protects its host, the woodland fly *Drosophila neotestacea*, against a virulent generalist parasitic nematode, *Howardula aaronymphium*. The prevalence of infection is very high in the wild, ranging from ~25-40%, and in symbiont-free flies, nematode infection results in sterility.

However, in flies that harbour *Spiroplasma*, fertility is fully restored. As a result, *Spiroplasma*-infected flies are at a selective advantage over their uninfected counterparts, and the infection is currently spreading across N. America. I will discuss the dynamics of *Spiroplasma* infection and spread, as well as its ecological and evolutionary consequences. I will also discuss our recent work attempting to understand how *Spiroplasma* provides protection, focusing on the characterization of a recently discovered *Spiroplasma*-encoded toxin.

PLENARY SESSION Monday 11:30 **3****No nematode is an island: Interactions between entomopathogenic nematodes and other organisms***Christine T. Griffin*

Biology Department, Maynooth University, Maynooth, County Kildare, Ireland

Correspondence: christine.griffin@nuim.ie

Entomopathogenic nematodes (EPN) represent a mutualistic association between nematode and bacterium capable of causing rapid death of insects. While some new EPN (*Osccheius* spp.) have been recently recognised, the best studied EPN are *Steinernema* spp. and *Heterorhabditis* spp. nematodes associated with the Enterobacteriaceae, *Xenorhabdus* spp. and *Photorhabdus* spp., respectively. The two associations share many features in common and are believed to have arrived independently at the entomopathogenic lifestyle. EPN are important models in studies of symbiosis and parasitism. There is a high degree of specificity in the relationship between each nematode and its bacterial partner, but it is becoming increasingly clear that a single nematode may utilise and even form natural associations with different symbionts, providing opportunity for niche expansion. When dispersing and host-finding, nematode infective juveniles may make use of other organisms – insects as phoretic hosts, and plant chemistry and architecture as guides to find root-feeding insects. Between them, nematode and bacteria kill insect hosts within days, and utilise the cadaver as a resource on which to multiply. However, insects are also a valuable resource for many other competing organisms. EPN have a variety of mechanisms to deter, kill or outgrow competitors, including production of antimicrobial compounds by the bacterial symbiont and physical attack by nematodes on competitors. Conspecific nematodes represent both competitors and potential collaborators (for reproduction and for overcoming difficult hosts); nematode transmission strategies are expected to be shaped by natural selection to optimise inclusive fitness. Despite nematodes' simple organisation, the complexity of their behaviour provides exciting challenges for multi-disciplinary investigation.

PLENARY SESSION Monday 12:00 **4****The ecology of virulence in insect associated bacteria: Field experiments and experimental evolution***Ben Raymond*

Imperial College London, Silwood Park campus, Ascot, Berks, SL5 7PY, UK

Correspondence: b.raymond@imperial.ac.uk

Bacillus thuringiensis invests a great deal of resources in producing diverse virulence factors during vegetative growth and at sporulation. However, for many decades *B. thuringiensis* had an uncertain reputation as a pathogen: the adaptive basis of investing in Cry toxins was questioned and it was suggested that *B. thuringiensis* might be a soil bacterium or a plant symbiont. Recent experiments have shown that reproduction of *Bt* is dependent on the death of its host. Moreover, presence of larval

hosts can increase the population size of *Bt* in its soil reservoir in the field, from where *Bt* is able to colonize plant tissues. In general, evolution of virulence theory can explain the link between investment in virulence and pathogen fitness. For *Bt*, and other insect parasites, theories that include kin selection and cooperation have proved particularly powerful in explaining investment in Cry toxins, vegetative virulence factors and in symbiont growth rate. Kin selection theory shows that the conflict between investing in traits that are individually costly but beneficial to groups, can be resolved if individuals tend to cooperate with bacteria that share their genes (high relatedness), a pattern shown by these entomopathogens in natural contexts. Indirect transmission via a soil reservoir, and cooperative conflicts can explain some of the ecological mysteries of *Bt*, such as the rarity of cadavers and epizootics in the field. Moreover, interrogating the relationship between pathogen fitness and virulence with evolutionary theory can have relevance for understanding how to maintain or improve the virulence of biocontrol agents.

SYMPOSIUM Monday 14:00 – 16:00
MICROSPORIDIA/ DISEASES OF BENEFICIAL INVERTEBRATES

Microsporidia as Emerging Pathogens

SYMPOSIUM CROSS-DIVISION Monday 14:00 **5**

The complex relationship between microsporidia and fungi

Patrick J. Keeling

Department of Botany, University of British Columbia, Vancouver
BC Canada V6T 1Z4

Correspondence: pkeeling@mail.ubc.ca

Our view of the position of microsporidia in the tree of eukaryotes has been substantially revised a number of times since they were first described many years ago. Identifying this position is not a trivial task, because they lack many typically 'eukaryotic' characteristics often used to compare one cell to another, and they are equally problematic at the molecular level due to the accelerated rate of sequence change in their genomes. Together, these features make it hard to compare microsporidia to other eukaryotes, and accordingly hard to understand how they relate to other lineages. Because biologists often, even subconsciously, rely on a phylogenetic framework to make functional predictions and reconstruct the evolutionary history of organisms, reconciling microsporidia with eukaryotic diversity is an important challenge and worth the effort. I will review the long and winding road to our current view of where microsporidia fall in the tree of eukaryotes, highlighting new advances that stem from a greater appreciation for the diversity of fungal related lineages. There are good reasons to be optimistic that we are near to understanding exactly how microsporidia fit into the tree of eukaryotes, which affects our understanding of the origin of novel characteristics of the lineage.

SYMPOSIUM CROSS-DIVISION Monday 14:20 **6**

Fish microsporidians: Emerging pathogens or emerging knowledge?

Mark A. Freeman

Institute for Experimental Pathology at Keldur, University of
Iceland, Reykjavik, Iceland

Correspondence: mf33bitw@gmail.com

Microsporidia are well-documented parasites of fish from both the marine and freshwater environments, with some genera

causing the formation of large xenoparasitic complexes which are characterised by an extensive hypertrophic growth of host cells, referred to as xenomas. Such obvious and intriguing infections have meant that fish-infecting microsporidians have received substantial research attention over the last 100 years. In addition, the relatively recent intensification of fish farming practices have meant that microsporidians as pathogens of farmed fish have become increasingly important and we now consider them as problematic pathogens in the global aquaculture industry. There are also concerns about food safety and the zoonotic potential of these opportunistic obligate parasites.

Here we consider our current understanding of fish microsporidians and further examine important aspects about these organisms, including host specificity, host-parasites interactions, lifecycles and identify particular species of interest; especially those that have recently emerged as potentially problematic pathogens for the aquaculture industry

SYMPOSIUM CROSS-DIVISION Monday 14:40 **7**

Understanding phylogenetic relationships among species in the *Nosema/Vairimorpha* clade: What does genetic similarity say about host switching in the microsporidia?

*Wei-Fone Huang*¹, *James Becnel*², *Leellen Solter*¹

¹Illinois Natural History Survey, Prairie Research Institute at the University of Illinois at Urbana-Champaign, IL, USA; ²United States Department of Agriculture, Agricultural Research Service, Gainesville, FL, USA

Correspondence: wfhuang@illinois.edu

Microsporidian species with high genetic identity based on rDNA phylogenies have been isolated from different insect species and even higher level host taxa across the globe. Complicating the picture, for genera such as the lepidopteran *Nosema* and *Vairimorpha*, isolates from different hosts may be morphologically identical while some species complexes include isolates that differ significantly in morphology, tissue tropism, host range and pathogenicity. Morphological characters such as octospore formation in the *Vairimorpha*, once thought to be apomorphies, cannot be used to separate these two genera or possibly even species. Difficulties in distinguishing isolates in turn complicates research on microsporidia suspected of host-switching to new and economically or ecologically important hosts. We used a combination of rRNA, HSP70, and RPB1 genes to evaluate and clarify the relationships among a large collection of species and isolates in the *Nosema/Vairimorpha* clade. Our data will be useful for classifying new isolates, describing species and evaluating the potential for microsporidia to invade new hosts.

SYMPOSIUM CROSS-DIVISION Monday 15:10 **8**

Emergent pathogens of invertebrates: Environmental sampling to identify novel parasite lineages

*Bryony A. P. Williams*¹, *Kristina Hamilton*¹, *David Bass*²

¹Biosciences, College of Life and Environmental Sciences, University of Exeter, Exeter, UK; ²Centre for Environment, Fisheries & Aquaculture Science, Weymouth, Dorset, UK

Correspondence: b.a.p.williams@exeter.ac.uk

Molecular surveys of environmental habitats using small subunit (SSU) ribosomal DNA environmental gene libraries now consistently uncover new microbial diversity and these new branches range in novelty from new subspecies to new branches in the tree of life equivalent to phyla. In some studies, parasitic cells make up a large proportion of this diversity, suggesting that protozoan parasites represent an important component of many ecosystems. However it can be challenging to fully sample

parasite diversity and distribution in the environment due to their changeable geographic and temporal distributions and the fact that they may be surrounded by a host that has to be sampled for the parasite DNA to be retrieved by the survey.

In this piece of work, we sample invertebrates across a temporal and geographic gradient in the UK to retrieve DNA from hosts and their associated parasites. We use a broad eukaryotic primer set to amplify the v9 region of the SSU gene from hosts and pathogens. Additionally we used a specific primers set to target the V4 region of SSU in the microsporidia. We analyse sequences retrieved through 454 sequencing, uncover novel potentially parasitic lineages in the microsporidia and in other eukaryotic lineages, and look for association patterns between these and invertebrates that may represent their hosts. This work aims to uncover novel diversity of microsporidia and other important pathogens, with a view to understanding the reservoir within the environment with the potential to cause emergent infection in economically important hosts.

SYMPOSIUM CROSS-DIVISION Monday 15:30 **9**

Investigations into the composition of the microsporidian polar tube

Louis M. Weiss, Kaya Ghosh, Bing Han

Departments of Medicine and Pathology, Albert Einstein College of Medicine, Bronx, NY, 10461, USA

Correspondence: louis.weiss@einstein.yu.edu

Microsporidia are remarkable parasites that have been studied for more than 150 years. They are remarkable in their exploitation of all animals ranging from cryptic, benign infections to spectacular, massive infections that cause extensive damage and often death of the host. These organisms produce spores with a unique invasion mechanism that are one of the most complex single celled forms known in the biological world. In humans infection, in either immune competent and immune deficient hosts, can result in diarrhea, encephalitis, myositis or conjunctivitis. These enigmatic protists are classified as NIH category B priority pathogens and EPA pathogens of interest as they are transmitted by both food and water sources. Besides human disease burdens, infection with microsporidia has major economic impacts on agriculture (via effects on insects and sericulture), aquaculture and animals (food, domestic and wildlife). The invasion mechanism used by these organisms is unique involving a specialized organelle, the polar tube and its associated structures. Despite the polar tube being recognized for over 100 years, the composition, structural biology and mechanism of interaction of the polar tube with its host cell during invasion are still unknown. A long standing research program in my laboratory group is focused on understanding the mechanism of invasion and the structural biology and composition of the polar tube. We have developed techniques for the purification of this structure, identified polar tube proteins and their post translational modifications and how these proteins interact. Furthermore, our studies have begun to define the functional roles of these proteins in the structural biology of the polar tube and the process of invasion. In other microbes studies on invasion have provided key data for understanding pathogenesis and for new therapeutic approaches to the management of infections. To this end, we have demonstrated that both antibodies to polar tube proteins and glycans related to the *o*-glycosylation seen on polar tube protein 1 can block invasion. We have recently characterized polar tube protein 4 from *E. hellem* (EhPTP4) using both proteomic and microscopic approaches. Immunofluorescence using antibodies to PTP4 demonstrated that this protein localizes to the end of polar tube suggesting it may be involved in the process of invasion. This protein provides the first marker for the site of invasion of the

host cell by the microsporidia. Binding assays confirmed EhPTP4 binding to RK13 and HFF cells. Analysis of the PTP4 demonstrates the presence of chitin binding, ricin and cellulose binding domains, and the presence of these various domains was confirmed by the ability of rEhPTP4 to bind chitin beads and inhibition of binding of rEhPTP4 to host cells by various monosaccharides. Co-immunoprecipitation (Co-IP) coupled to proteomic analysis has identified a host cell surface membrane protein as a potential interacting protein for EhPTP4. Information gained by delineating, in detail, the function and components of the invasion organelle should lead to new and novel interventions that could limit or interdict the transmission of these emerging pathogens.

CONTRIBUTED PAPERS Monday 14:00 – 16:00

Bacteria 1

CONTRIBUTED PAPER Monday 14:00 **10**

Resistance to *Bacillus thuringiensis* Cry2Ab toxin in *Helicoverpa* spp. is conferred by mutations in a novel ABC transporter

Wee Tek Tay¹, Rod J. Mahan¹, Thomas K. Walsh¹, Sharon Downes², William J. James¹, Siu Fai Lee³, Annette Reineke⁴, Adam K.

Williams³, Karl J. H. Gordon¹, David G. Hecke⁵

¹CSIRO, Black Mountain Laboratories, Canberra, ACT, Australia;

²CSIRO, Australian Cotton Research Institute, Narrabri, NSW, Australia; ³Department of Genetics, University of Melbourne, Parkville, VIC, Australia; ⁴Institute for Phytomedicine, Geisenheim University, Geisenheim, Germany; ⁵Department of Entomology, Max Planck Institute for Chemical Ecology, Jena, Germany

Address for correspondence: heckel@ice.mpg.de

Transgenic crops expressing the insecticidal protein Cry2Ab from *Bacillus thuringiensis* (Bt) are used worldwide to suppress damage by lepidopteran pests, pyramided with Cry1Ac toxin to delay resistance evolution. Previous studies have shown that Cry2Ab toxin-binding sites in the midgut are different from Cry1Ac-binding sites, but little additional information on Cry2Ab mode of action is available. Resistance to Cry1Ac can be caused by alterations in its binding targets, including a membrane-bound alkaline phosphatase, a 12-cadherin-domain protein, and the ABC transporter ABCC2, but resistance mechanisms to Cry2Ab have not been identified. In Australia, Cry2Ab-resistant strains of *Helicoverpa armigera* and *H. punctigera* have been isolated from the field using F₁ and F₂ screens, and these strains show no cross-resistance to Cry1Ac. Using a positional cloning approach, we identified mutations in a novel ABC protein in both species that truncate the protein and confer high levels of resistance to Cry2Ab. This resistance is genetically independent from Cry1Ac resistance caused by mutations or downregulation of a different ABC protein, ABCC2. We discuss the implications of this finding for Bt toxin modes of action and resistance management.

CONTRIBUTED PAPER Monday 14:15 **11-STU**

Functional range of insect ABCC transporters as a Cry toxin receptor

Haruka Endo^{1,2}, Shiho Tanaka¹, Ryoichi Sato¹

¹Graduate School of Bio-Applications and Systems Engineering, Tokyo University of Agriculture and Technology, Koganei, Tokyo, Japan; ²Research Fellow of Japan Society for the Promotion of Science

Correspondence: ryoichi@cc.tuat.ac.jp

Cry toxins produced by *Bacillus thuringiensis* have specific toxicity for target insects. Although the specificity for target insects seems

to largely depend on receptor interaction, it is still unclear which receptor(s) is/are involved in the specificity of each Cry toxin subfamily. ATP-binding cassette transporter C2 (ABCC2) in lepidopteran insects is a receptor of Cry1A toxins. Recently, ABCC3— a Paralog of lepidopteran ABCC2— was also reported to function as a receptor of Cry1 toxins, indicating the possibility that other ABCC transporters may function as Cry toxin receptors. In the present study, to clarify functional range of ABCC transporter as a receptor of Cry1A toxin, we analyzed cell swelling-inducing activity of insect ABCC transporters, which are close relative of ABCC2. Phylogenetic analysis among arthropods ABCC transporters tentatively showed that ABCC2 and ABCC3 are lepidopteran-specific and that non-target insects of Cry1A toxins such as dipteran and coleopteran insects have no members in these two clades. ABCC2 and ABCC3 in lepidopteran insects including *Bombyx mori* and *Spodoptera exigua* conferred Cry1A toxin-susceptibility to cultured cells. However, the most closely related ABCC transporters of ABCC2 in *Tribolium castaneum* and *Aedes albopictus* did not function as Cry1A receptors. These results suggested that the target specificity of Cry1A toxins for lepidopteran insects largely depends on the two ABCC transporters uniquely found in lepidopteran insects. We will report results from further analysis on receptor function of ABCC3 and discuss a role of ABCC transporters in determining specificity of Cry toxins.

CONTRIBUTED PAPER Monday 14:30 **12-STU**

Cry1A toxins cause rapid cell lysis in a clonal stable non-lytic expression system, expressing ABC-C2 and cadherin

Anne Karpinski, Yannick Pauchet, David Heckel

Department of Entomology, Max Planck Institute for Chemical Ecology, Jena, Germany

correspondence: akarpinski@ice.mpg.de

Pest insect resistance to insecticidal crystal (Cry) proteins from *Bacillus thuringiensis* (Bt) is evolving due to their increasing usage in agriculture. Two genes (a 12-domain Cadherin protein and an ABC transporter) have been genetically linked to Cry toxin resistance in *Heliothis virescens*. Even though the Cry1A toxin mode of action is quite well understood, the specific role of ABC-C2 and its importance for toxicity still need further investigation. Non-lytic stable clonal Sf9 cell lines were created as follows: one cell line expressing only the Cadherin protein, one expressing only the ABC-C2 transporter, and one expressing both Cadherin and ABC-C2 from *H. virescens*. The absence of endogenous Cadherin, ABC-C2 and aminopeptidase N (APN) from the transcriptome of Sf9 cells was crucial for the success of our experiments. Toxicity of Cry1A proteins (Cry1Aa, Cry1Ab, Cry1Ac) can be observed in doubly transfected cells in which up to 86% of the cells died after 8 h treatment. Although cell swelling was observed in the cell line expressing only ABC-C2 and in the one expressing both Cadherin and ABC-C2, rapid lysis and death within 30 min after Cry1Ac treatment could only be observed for the doubly transfected cells. No such effect could be observed in control Sf9 cells or in cells only expressing the Cadherin. Our findings underline the central role played by ABC-C2 in the Cry1A toxin mode of action and resistance in *H. virescens*, but also emphasize that ABC-C2 and Cadherin interact to increase Cry1A toxicity.

CONTRIBUTED PAPER Monday 14:45 **13**

Expressing a lepidopteran ABCC2 gene in transgenic *Drosophila* causes Bt Cry1Ac susceptibility without requiring a cadherin-like protein receptor

Tristan Stevens, Simon W. Baxter

School of Biological Sciences, University of Adelaide, Australia

Correspondence: simon.baxter@adelaide.edu.au

The diamondback moth, *Plutella xylostella*, is a serious agricultural pest of *Brassica* crops worldwide and can be extremely difficult to control. Populations commonly evolve resistance to insecticides used against them, including Bt Crystal (Cry) toxins produced by the bacteria *Bacillus thuringiensis*. Hundreds of different Cry toxins have been described, and many are highly specific to targeted pests yet benign to non-target organisms. For example, Cry1Ac kills most lepidopteran moth pests but not flies, such as *Drosophila*.

The precise mode of action of Bt toxins remains controversial, as many midgut receptors and pathways to insect death have been identified. A lepidopteran cadherin-like protein is central to several models, as either a toxin receptor or protoxin activator. Mutations in the diamondback moth ABCC2 gene, rather than a cadherin-like protein, were previously associated with Cry1Ac resistance. As *Drosophila* is not susceptible to Cry1Ac and lacks a Cadherin-like protein orthologue, we used this insect model to investigate whether the diamondback moth ABCC2 protein acts as a functional toxin receptor. The moth ABCC2 gene was fused with a GFP reporter gene, cloned into the pUAST vector and transgenic *Drosophila* lines generated. Using the GAL4/UAS system, we observed successful expression of the ABCC2-GFP construct through GFP localization to cell membranes. When fed with artificial diet containing trypsin activated Cry1Ac toxin, or Cry1Ac protoxin, larvae expressing ABCC2 in the midgut became highly susceptible. This data shows ABCC2 acts as a Cry1Ac receptor, and toxin activation and mortality can be achieved without the Cadherin-like protein.

CONTRIBUTED PAPER Monday 15:00 **14**

Differential toxicity of Cry1Ca and Cry1Ac to *Spodoptera exigua* (Lepidoptera: Noctuidae)

Younqjin Park¹, Salvador Herrero², Yonggyun Kim¹

¹Department of Bioresource Sciences, Andong National University, Andong 760-749, Korea; ²Department of Genetics, Universitat de València, Dr Moliner 50, 46100 Burjassot, Spain

Correspondence: happy2pyji@gmail.com

Cry toxins produced by an entomopathogenic bacterium, *Bacillus thuringiensis* (Bt), are pathogenic factors specific to insect species. A serious lepidopteran pest, *Spodoptera exigua*, is highly susceptible to Cry1Ca, but much less to Cry1Ac. This study investigated the differential toxicity with respect to their differential affinities to a Bt receptor, cadherin. RNA interference (RNAi) of a cadherin of *S. exigua* (SeCad1) significantly suppressed the Cry1Ca to the toxic level of Cry1Ac. Binding affinity of Cry1Ca to brush border membrane vesicle (BBMV) of *S. exigua* midgut was significantly lost after SeCad1 RNAi. Binding affinity of Cry1Ac to BBMV was much low compared to that of Cry1Ca and less sensitive to SeCad1 RNAi. Direct binding assay of Cry toxins to SeCad1 was assessed using a recombinant cadherin repeat 10-11 (CR10-11) of SeCad1. The addition of CR10-11 to Cry1Ca significantly enhanced the toxicity under SeCad1 RNAi. However, the synergistic effect of CR10-11 on toxicity of Cry1Ac was not much significant. Binding assay of Cry toxins to CR10-11 explained the poor binding affinity of Cry1Ac compared to Cry1Ca. These results indicate that the differential toxicity of Cry toxins against *S.*

exigua is caused by the different binding affinities to the cadherin Bt receptor.

CONTRIBUTED PAPER Monday 15:15 **15-STU**

Models for the interaction between cadherin-like receptor BT-R1 and three Cry1A family toxins from *Bacillus thuringiensis*

Dioqo Martins-de-Sa¹, Fernando C. A. Fonseca², Wagner A. Lucena², Patricia B. Pelegrini², Maria Fatima Grossi-de-Sa²

¹Cellular Biology Department, University of Brasília, Brasília, Brazil;

²Embrapa Genetic Resources and Biotechnology, Brasília, Brazil

Correspondence: dmartinsdesa@gmail.com

Cry1Aa, Cry1Ab, and Cry1Ac present 82 to 90% amino acid identity and are toxic to *Manduca sexta* larvae. Extensive substitution of loop residues in domain II suggests that this region is responsible for specific binding to receptor. The interaction with cadherin-like receptors has been described as an important step for the correct removal of helix α 1 in domain I and subsequent events leading to the insect's death. After homology modeling and a selective protein docking, two models describing the interactions of Cry1Ab to the *M. sexta* BT-R₁ receptor were assessed using molecular dynamics simulations. Twelve binding regions were identified for each protein and their biophysical properties were further evaluated. To validate our model, we synthesized peptides corresponding to each region. Preliminary result for one model show that loop 3, notorious for receptor recognition, binds a region previously unidentified in *Manduca sexta* cadherin-like receptor. This new toxin binding region shows the same hydrophobicity profile of an antibody epitope previously described to bind specifically to loop 3. Most interestingly, binding occurs with over 266-fold less peptide concentration in pH 9.0 than in pH 7.4. The physiological pH in the insect midgut is approximately 9.0, which corroborates that at least one of the models reproduces *in vivo* interaction. Ongoing work will show if both models are plausible to occur, or if one of them is preferable to the other. Overall, these models allowed the observation of the toxin's behavior when binding to BT-R₁ and help explain many *in vitro* experiments concerning Cry1A and cadherin-like receptors.

CONTRIBUTED PAPER Monday 15:30 **16**

A high-throughput, *in vitro* assay for *Bacillus thuringiensis* Cry proteins

Michi Izumi Wilcoxon, Jaclyn Dennis, Sabina Lau, Weiping Xie, You You, Song Leng, Ryan Fong, Takashi Yamamoto

Plant Protection, Ag Biotechnology, DuPont Pioneer, Hayward, CA, USA

Correspondence: michi.izumi@pioneer.com

A high-throughput, *in vitro* assay for *Bacillus thuringiensis* (Bt) Cry proteins was developed and evaluated for screening a large number of Cry protein variants produced by DNA shuffling. This automation-amenable assay exploits an insect cell line expressing a single receptor of Bt Cry proteins. Cell mortality caused by the activated Bt Cry toxin was determined by chemical cell viability assay in 96/384-well microtiter plates utilizing CellTiter 96[®] obtained from Promega. A widely-accepted mode-of-action theory of certain Bt Cry proteins suggests that the activated toxin binds to one or more receptors and forms a pore through the insect gut epithelial cell apical membrane. A number of insect proteins such as cadherin-like protein (Cad), aminopeptidase-N (APN), alkaline phosphatase (ALP) and ABC transporter (ABCC) have been identified as the receptors of Bt Cry toxins. In this study, Bt Cry toxin receptors, *Ostrinia nubilalis* (European corn borer) cadherin-like protein (On-Cad) and aminopeptidase-N 1 and 3 (On-APN1, On-APN3) and Spodoptera frugiperda (fall

armyworm) cadherin-like protein (Sf-Cad), were cloned in an insect cell line, Sf21, and a mammalian cell line, Expi293F. The sensitivity of the recombinant cells to the toxin was tested against a variant of Cry1Ab protein called IP1-88 which was produced previously by DNA shuffling.

CONTRIBUTED PAPER Monday 15:45 **17**

Transcriptome response of *Aedes aegypti* against *Bacillus thuringiensis* and the pathway for enhancing the mosquitocidal activity

Wu Songqing^{1,2}, Liu Zhaoxia², Wang Yafang², Xu Zhuoying², Guo Yajie², Xiong Yueting², Mou Yani², Zhu Xiaoli², Zhang Feiping¹, Zou Shuangquan¹, Zhang Lingling², Guan Xiong²

¹Collage of Forestry, Fujian Agriculture and Forestry University, Fuzhou, 350002, People's Republic of China; ²Key Laboratory of Biopesticide and Chemical Biology, Ministry of Education, Fujian Agriculture and Forestry University, Fuzhou, 350002, People's Republic of China

Correspondence: guanxfafu@126.com, fpzhang1@163.com

It has been estimated that over one million people worldwide die from mosquito transmitted diseases every year. Bti is one of the few *Bacillus thuringiensis* strains that are highly pathogenic to mosquito larvae. But application of this insecticide is minor, because of the high cost and relative lower mosquitocidal activity, as compare with conventional pesticides. Therefore, how to enhance the mosquitocide efficacy and reduce the cost of Bti insecticides has been a difficult issue which we are confronted. Understanding of Bti-mosquito interactions will help to develop new Bti toxins for mosquitoes and mosquito-borne disease management. To find some biotechnological applications for enhancing the activity of Bti toxins against mosquitoes, this study investigate mosquito-Bti interactions at both cellular and molecular levels. *Ae. aegypti* transcript responses to experimental challenge with mosquitocidal strain *B. thuringiensis* subsp. *israelensis* LLP29 has been analyzed with next generation sequencing. Three candidate genes, have been selected based on their differential expression as representatives of the different functional categories to perform gene silencing by RNA interference and analyze their functional role. Detoxification enzymes assay revealed that the exposure of Bti significantly affect the activities of amylase, cytochromes P450, Na⁺/K⁺-ATPase, acetylcholinesterase and Glutathione S-transferase. Cost-effective methods, by using spent mushroom substrate as raw material for Bti cultivation, have been established for the mass production of *Bacillus thuringiensis* by solid and liquid state fermentation. Results from this study will pave a scientific way for the future development of novel cost-effective anti-mosquito biological agents for mosquito pest management.

CONTRIBUTED PAPERS Monday 14:00 – 16:00

Microbial Control

CONTRIBUTED PAPER Monday 14:00 **18**

Autodissemination strategy for field suppression of *Bactrocera dorsalis* (Diptera: Tephritidae) using *Metarhizium anisopliae*-based biopesticide on mango

Sunday Ekesi, Nguya K. Maniania

International Centre of Insect Physiology and Ecology (*icipe*), Nairobi, Kenya

Correspondence: sekési@icipe.org

Bactrocera dorsalis is a major quarantine pest of horticulture in Africa and elsewhere attacking over 30 host plant species, although mango is the preferred host. Direct damage on mango has been reported to range from 30-80%. In a field experiment conducted over 2 seasons in Malindi, Kenya, we evaluated the use of autodissemination strategy with the entomopathogenic fungus, *Metarhizium anisopliae* ICIPE 69, for the suppression of *B. dorsalis* and the impact on fruit infestation and yield. Velvet-coated Lynfield traps were used as autoinoculators and laced with methyl eugenol on cotton-wick (as attractant) and 0.5 g of conidia of *M. anisopliae*. The autoinoculators were deployed at the rate of 30 traps ha⁻¹ in orchards assigned to biopesticide treatment. Control orchards were left untreated. In 2011, the biopesticide treatment reduced *B. dorsalis* catches relative to the control by 79.1% within 4 weeks and 94.6% within 8 weeks. Mean mortality of adult fruit flies mycotized as a result of *M. anisopliae* infection ranged from 54.2-76.5% in male flies and 32.2-61.8% in females. The fungus persisted for 3-4 weeks in the autoinoculator and declined significantly thereafter. At harvest, the proportion of fruit infested was significantly lower in treated orchards (6.4%) compared to control orchards (61.7%). Mango yield was significantly higher in orchards assigned to biopesticide (11,802 kg ha⁻¹) compared to control orchards (2,911 kg ha⁻¹). Similar results were obtained in 2012 season, indicating that autodissemination strategy using *M. anisopliae*-based biopesticide holds great promise for suppressing fruit flies and increasing yield in mango orchards.

CONTRIBUTED PAPER Monday 14:15 **19**

Development of granules based on the biomass of liquid fermented *Metarhizium anisopliae*, *Isaria fumosorosea* or *Beauveria bassiana* for control of soil dwelling pests

*Dietrich Stephan*¹, *Medea Buranjadze*², *Tanja Bernhardt*¹, *Juliana Pelz*¹, *Johannes Schäfer*¹, *Christopher Seib*¹

¹JKI-Institute for Biological Control, Heinrichstrasse 243, 64287 Darmstadt, Germany; ²Agricultural University of Georgia, 240,

D.Agmashenebely Alley, Tbilisi 0159, Georgia

Correspondence: dietrich.stephan@jki.bund.de

Although entomopathogenic fungi can be grown on solid media the fermentation time in solid state fermenter is long and labor intensive. Therefore, we investigated the possibility of formulating granules based on liquid fermented biomass of the entomopathogenic fungi *Metarhizium brunneum* (strain JKI-BI-1339 = Ma43), *Beauveria bassiana* (strain Bba 007) and *Isaria fumosorosea* (strain JKI-BI-1496).

After fermentation the biomass was filtered to separate the filtrate, including submerged spores, from the rest of the biomass. After filtration submerged spores can be used for the development of sprayable formulations. The filtercake including mycelium and submerged spores was used for the production of granules using fluid bed drying. To analyze this, experiments on the thermal tolerance at temperatures of 25 °C, 50 °C and 70 °C were conducted firstly in a water bath and a compartment dryer. This was necessary to verify whether the fungi survive at a temperature of 50 °C when using fluid bed drying. Because the tested fungi survived these temperatures granules were produced by coating millet with a strain specific biomass concentration. The sporulation on the granule was investigated and furthermore the influence of the soil moisture on the fungal growth. E.g. strain JKI-BI-1496 was able to grow out and sporulate at all tested residual moistures of the soil (3, 20, 30, 40 & 45 % rh). The highest sporulation was achieved for a residual moisture of 40 % rh. Further optimization steps and the practicability of biomass based formulations will be discussed.

CONTRIBUTED PAPER Monday 14:30 **20-STU**

Can pheromone enhance the transmission of *Metarhizium brunneum* in *Agriotes obscurus* click beetles?

*Joyce P. S. Leung*¹, *Jenny S. Cory*¹, *J. Todd Kabaluk*², *Alida F. Janmaat*³

¹Department of Biological Sciences, Simon Fraser University, Burnaby, BC, Canada; ²Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, Agassiz, BC Canada; ³Department of Biology, University of the Fraser Valley, Abbotsford, BC, Canada

Correspondence: jpl15@sfu.ca

Pheromone, when used in combination with microbial control agents, has been primarily used as an attractant in attract-and-kill strategies. However, in addition to being an attractant, pheromones can also alter insect movements and social interactions. We investigated the potential of female sex pheromone granules in enhancing the transmission of the fungal pathogen *Metarhizium brunneum* in *Agriotes obscurus* (click beetles), of which the larvae, wireworms, are a pest of many root crops in North American and Europe. Using video tracking, we investigated how click beetle behaviour changed in the presence of pheromone. We then began to link how these behavioural changes can impact *M. brunneum* transmission through horizontal transmission and conidia pickup from the environment. Enhancement of pathogen epizootic potential could be a step in shifting microbial control practices from an inundative approach to one that is inoculative, where management of a pest is beyond the short term.

CONTRIBUTED PAPER Monday 14:45 **21**

Less effort - more efficient: An attract and kill strategy for wireworm control in potato

*Mario Schumann*¹, *Anant Patel*², *Stefan Vidal*¹

¹Georg-August-University Goettingen, Department of Crop Sciences, Goettingen, Germany; ²University of Applied Sciences, Department of Engineering and Mathematics, Bielefeld, Germany

Correspondence: svidal@gwdg.de

In recent years wireworms became the most important herbivorous pest species in potato production systems in many parts of Europe. The larvae feed on the tubers before harvest, causing qualitative damage and paving the way for secondary bacterial or fungal infections, both resulting in reduced income for farmers. These yield losses are of concern for both organic and conventional growers. Control options targeting wireworms are limited, due to the phasing out of effective insecticides; new control options are therefore desperately needed.

Wireworms use carbon dioxide gradients, established by growing roots, to locate their host plants. This long distance orientation cue is complemented by additional specific volatile cues emitted by the plants, resulting in a final acceptance of host plants for feeding.

Control strategies using biocontrol agents, such as entomopathogenic fungi, depend on high concentrations of spores per m², set against competing microorganisms in the rhizosphere, targeting the pest species. However, in addition to high costs control efficacies are generally low.

The "attract and kill" approach (A&K) turns this strategy upside down: instead of bringing the control agent to the larvae, we attract them to the control agent by combining the agent with capsules emitting CO₂. Wireworm mortality can significantly increase because of a more frequent contact with the control agent. To make this strategy work under field conditions, the capsules need to fulfill prerequisites, such as building up CO₂

gradients significantly higher than the background CO₂ concentrations in the soil for at least several weeks.

Lab experiments demonstrated that wireworms were attracted to and fed on these artificial CO₂-emitting capsules. In the field we combined these capsules with an isolate of an entomopathogenic fungus, acting as the kill component. Treatments were applied into the potato dams, either below or between the tubers at two different dates during the growing season. We used fields with a high incidence of wireworm damage in previous years. Application of the A&K capsules resulted in significantly lower tuber damage in most, but not all fields, depending on the treatment schedule. Late season application of the A&K capsules did not prevent damage by wireworms because mortality did not immediately occur following contact with the killing agent, thus feeding damage was still observed.

Necessary improvements of the A&K strategy for a standardized application routine in the field are currently tested in field experiments at several locations. The implementation roadmap for this strategy will be discussed.

CONTRIBUTED PAPER Monday 15:00 **22**

***Drosophila suzukii* fungal infections and transmissions: A potential field suppression strategy**

Joan Cossentine, Mairi Robertson

Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Summerland, BC, Canada

Correspondence: joan.cossentine@agr.gc.ca

Drosophila suzukii adults were found to acquire lethal fungal infections in the laboratory through surface-treated contact with isolates of *Metarhizium brunneum*, *Beauveria bassiana*, *Isaria fumosoroseus* and *Lecanicillium lecanii*. In these trials, the *M. brunneum* F52 isolate had the greatest impact on the flies, followed by the *B. bassiana* GHA isolate. Scanning electron microscopy confirmed that conidia accumulated on *D. suzukii* pretarsi and labella after exposure to the treated surfaces and horizontal transmission of *M. brunneum* between flies was verified. Although *D. suzukii* longevity decreases with increasing temperatures, the flies generally survive for two to three weeks in the field and *M. brunneum*-induced mortality in the laboratory began 5 to 7 days post conidia exposure. Significantly lower F1 counts were recorded over 14 d from *D. suzukii* females exposed to *M. brunneum* in the laboratory; a decrease that was attributed to mortality over time. Based on these findings, it appears that field suppression of the new North American and European invasive pest may be possible through the incorporation of *Metarhizium* conidia into a bait or surface treatment.

CONTRIBUTED PAPER Monday 15:15 **23**

Ant diseases: A neglected area in insect pathology?

Roberto M. Pereira

Urban Entomology Lab., Entomology & Nematology Dept., University of Florida, Gainesville, FL, USA

Correspondence: rpereira@ufl.edu

Despite representing a considerable portion of the biomass on earth, their importance in many ecosystems, and role as pests in urban and agricultural systems, ants are relatively little studied for their pathology. With increasing number of ant species considered exotic pests in different parts of the globe, there is growing interest in the identification of ant pathogens that can be used in the microbial control of ant species. Indications from casual ant disease collections and more deliberate efforts point to a large number of diseases affecting Formicidae. However, relatively few ant pathogens have been studied sufficiently and/or

exploited for control of ant pests. Fungal and protozoan pathogens have been identified from several ant species, while viruses are only known from *Solenopsis* spp. Bacteria associated with ants and their nests have been isolated but no effective bacterial pathogens have been identified for use in control efforts. Most pathogens described from Formicidae form obvious outgrowths or significant changes in behaviors normally associated with ants. However, other diseases seem to have little effect on ant behavior, and have been identified from foraging ants captured in pitfall traps or other capture methods favoring normal active ants. Several pathogens already identified from ants await further investigations on their effects on ant populations, complete description of life cycle, and other aspects that may lead to better understand of the pathogen-ant relationship and potential use in microbial control of ants.

CONTRIBUTED PAPER Monday 15:30 **24**

The importance of searching for appropriate strains for the control of leaf-cutting ants

Patricia J. Folgarait, Daniela Goffré

Laboratorio de Hormigas, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Bernal, Buenos Aires, Argentina

Correspondence: patricia.folgarait@gmail.com

Leaf-cutting ants are important pests of pastures, plantations and several crops in Latin America. Nowadays there is a pressing need for replacing pesticides by alternative –environmentally safe– means of control. One of those within the biological type of control is the use of entomopathogens. It is very common to use collection strains or species found in the soil. Here, we compared the virulence of three different strains of *Purpureocillium lilacinum* when *Acromyrmex* spp. colonies were inoculated. We also tested the concentration of conidia that remained in the ant's body at increasing concentrations of inoculation. Finally, we tested the horizontal transmission from inoculated ants towards non-inoculated ones. Our results showed the importance of testing several strains, as they exhibited different degrees of virulence; the need for testing across many individuals from several nests, due to the innate dissimilarities found in colony responses; the relevance of knowing at which conidia concentration ants' bodies' saturated, as ants cannot carry and/or maintain the same concentrations as inoculated; that inoculated ants can transmit *P. lilacinum* conidia towards non-infected ants and produce their death; and finally, the relevance of testing the strains in more than one species if the pest is a species complex, as is the case with leaf-cutting ants. We advocate that the characteristics mentioned should be considered when choosing appropriate entomopathogenic strains for pest control.

SYMPOSIUM CROSS-DIVISION Monday 16 :30-18 :30
FUNGI/MICROBIAL CONTROL

**The (Underestimated) Value of Applied Research:
Moving the Theoretical to the Practical**

SYMPOSIUM PAPER Monday 16:30 **25**

Why biopesticides sometimes fail

Roma L. Gwynn¹, Michael Brownbridge², Travis R. Glare³

¹ Rationale, 1 Lintlaw Farm Cottages, Duns TD11 3QA, Scotland

² Vineland Research and Innovation, 4890 Victoria Avenue North, Box 4000, Vineland Station ON, LOR 2E0, Canada

⁴ Bio-Protection Research Centre, PO Box 85084, Lincoln University, Lincoln 7647, New Zealand

Correspondence: rgwynn@biorationale.co.uk

Biopesticides are increasingly used in crop protection for pest management in protected and field situations across a range of crops. They are used as stand-alone products, essential components of resistance management or as components of Integrated Pest Management systems where functional compatibility with other biological control agents is essential. However, micro-organism based biopesticides have a checkered history in terms of reproducible efficacy in the field. Some efficacy issues are more perception than reality, but the (apparent) lack of consistent effectiveness or 'low' efficacy can limit their uptake. What causes inconsistency after they have been properly delivered to the target in the crop? To what extent is the lack of consistency due to the innate nature of using living organisms and how much can be attributed to other factors? What roles do agro and microbial ecology play in the success of biopesticides after delivery? How do we measure this and then improve performance? This talk will place efficacy of applied biopesticides in an ecological context.

SYMPOSIUM PAPER Monday 16:54 **26**

Multiple roles so what should we measure? An ecological approach to promote the contribution of fungal entomopathogens in pest management within the agro-ecosystem

Nicolaj V. Meyling

Department of Plant and Environmental Sciences, University of Copenhagen, 1871 Frederiksberg C, Denmark
Correspondence: nvm@plen.ku.dk

Although often referred to as biopesticides, microbials applied for pest control are living organisms and their function and contribution in pest management systems should be considered from an ecological perspective rather than as the equivalent of chemical pesticides, i.e. spray-and-kill. Recent accumulated knowledge about the natural occurrence and distribution of fungal entomopathogens in agro-ecosystems, the factors governing the abundance of these fungi as well as new evidence for their ecological roles and functions will be valuable to promote the effects of these fungi when applied for pest control. Relevant examples will be presented and discussed in relation to application of fungal entomopathogens to elucidate how an ecological approach could promote the contribution of these fungi for pest management, how we could measure the effects, and what additional potential effects besides control of the target pest we could expect.

SYMPOSIUM PAPER Monday 17:18 **27**

Research and development of biological crop protection products – Bridging the gaps from discovery to field

Reed N. Royalty, Denise C. Manker

Bayer CropScience, Biologics. 890 Embarcadero Drive, West Sacramento, CA, USA 95605
Correspondence: reed.royalty@bayer.com

Basic agrichemical manufacturers are increasingly active participants in the biological crop protection space. A benefit of this participation is a substantial increase in R&D investment in biologics. This investment undoubtedly will result in the development and launch of more biologics products, but return-on-investment expectations means that these new products must deliver excellent and consistent in-field efficacy against major pests in major crops globally.

Bayer CropScience (BCS) approaches the challenge of developing profitable biologics that deliver excellent in-field performance at

two levels. Prior to field testing, an R&D platform is being implemented that leverages three key components: 1) an understanding of the relationship between the crop, the pest, the biological, and the environment, 2) the development of enabling technologies that will improve greatly the performance, and 3) the R&D processes and benchmarks that BCS has used successfully to develop highly effective and safe synthetic crop protection products.

After biological development candidates move into large-scale field testing, BCS uses the attribute definition done in the lab and greenhouse to test the candidates in a manner that will define accurately their commercial potential. Integrated crop solutions are created globally and locally; these solutions capitalize on the strengths and minimize the deficiencies of the biologics. Appropriate field-trial design and the magnitude of BCS field research capabilities are leveraged to permit the robust testing necessary to validate these integrated solutions.

SYMPOSIUM PAPER Monday 17:42 **28**

Adapting field trials for microorganisms – in practice

Edith Ladurner¹, Massimo Benuzzi¹, Sergio Franceschini²

¹CBC (Europe) S.r.l. – AREA TECNICA, via Calcinaro 2085 int. 7, 47521 Cesena (FC), Italy

²CBC (Europe) S.r.l. – BIOGARD Division, via XXV Aprile 44, 24050 Grassobbio (BG), Italy

Correspondence: eladurner@cbceurope.it

Micro-organisms are becoming increasingly important in integrated crop protection systems, because, in addition to being valuable pest control tools, they can help to reduce the risk of the development of pathogen strains or pest populations resistant to conventional plant protection products and of undesired residue levels in the final production. In order to be placed on the market as plant protection products, their efficacy against pests and diseases must be proven by following official guidelines, developed for conventional pesticides. However, by their nature, products based on micro-organisms may be highly specific in the pests or in the stage of the target pest they affect, and they may require specific conditions to reach acceptable efficacy. In many cases optimal efficacy can only be achieved when they are used as part of an integrated control strategy rather than as stand-alone products. This talk will provide examples on how field trials can be adapted for micro-organisms, and on how the experience of field trials can be transferred into practice for effective crop protection.

SYMPOSIUM PAPER Monday 18:06 **29**

How do we improve efficacy monitoring of biopesticides?

Travis R. Glare¹, Michael Brownbridge², Roma L. Gwynn³

¹Bio-Protection Research Centre, PO Box 85084, Lincoln University, Lincoln 7647, New Zealand

²Vineland Research and Innovation Centre, 4890 Victoria Avenue North, Box 4000, Vineland Station ON, L0R 2E0, Canada

³Rationale, 1 Lintlaw Farm Cottages, Duns TD11 3QA, Scotland

Correspondence: travis.glare@lincoln.ac.nz

To fulfil the predicted increasing use of biopesticides, these products must have demonstrated and reliable efficacy in the field. As will be discussed in the symposium, the perceived lack of reliable field efficacy has limited uptake of biopesticides. The actual efficacy of these products is likely to be much higher than the current methods of measurement suggest, given their multiple roles in the ecosystem. Our understanding of the complex interactions of biological agents after application suggest

we underestimate efficacy by measuring only direct impacts. How can we better measure 'efficacy' of these micro-organisms, which may be lower than the chemicals they are compared to in terms of direct pest mortality, and how do we need to change perceptions and pest management programs to accommodate this? Is the way forward to consider efficacy in terms of population ecology? This talk will consider these questions in light of the information presented in the symposium and offer some suggestions for the future.

CONTRIBUTED PAPERS Monday 16:30 – 18:30

Viruses 1

CONTRIBUTED PAPER Monday 16:30 **30**

Overexpression of two microRNAs enhances the infectivity of Sindbis virus TE5'2J strain in mosquitoes

Ning Huang¹, Jessica Moffitt¹, Kayvan Etebari², Sassan Asgari², Alexander W.E. Franz³, A. Lorena Passarelli², Rollie J. Clem¹

¹Division of Biology, Kansas State University, Manhattan, KS USA;

²School of Biological Sciences, University of Queensland, St. Lucia, Queensland, Australia; ³Department of Veterinary Pathology, University of Missouri, Columbia, MO USA

Correspondence: rclem@ksu.edu

The mosquito *Aedes aegypti* serves as a natural vector for chikungunya, dengue, and yellow fever viruses. In the laboratory, *A. aegypti* is also susceptible to infection by the model togavirus Sindbis virus (SINV). To be transmitted, these viruses must infect and replicate in the mosquito midgut and then escape from the midgut and infect salivary glands. In order to understand the potential role of caspases in midgut escape of Sindbis virus (SINV) during infection of *A. aegypti*, recombinant SINV strains were constructed that express CASPS19, an effector caspase from *A. aegypti*. Overexpression of CASPS19 in TE5'2J, a cell culture-adapted SINV strain with poor infectivity in *A. aegypti* mosquitoes, increased infection rates by several fold. However, a frame shift mutation eliminating the production of CASPS19 protein did not affect the enhancement of infection. This suggested the possibility that one or more small RNAs encoded within the *casps19* cDNA sequence were responsible for enhancing infection. Inspection of the *casps19* sequence indicated the presence of two potential microRNA (miRNA) coding sequences. Deletion of either putative miRNA coding sequence had no effect, but deletion of both abolished enhancement of infectivity. Both miRNAs were shown to be naturally expressed in *Ae. aegypti* midgut. Experiments are underway, using mimics and inhibitors of these miRNAs, to further study their ability to enhance infectivity and to determine their targets.

CONTRIBUTED PAPER Monday 16:45 **31**

Microbial challenges and stress modify piRNA cluster abundance in the dengue mosquito, *Aedes aegypti*

Kayvan Etebari¹, Mazhar Hussain¹, Gregor J. Devine², Sassan Asgari¹

¹Australian Infectious Disease Research Centre, School of Biological Sciences, The University of Queensland, Brisbane QLD 4072, Australia; ²QIMR Berghofer Institute of Medical Research, Brisbane, QLD 4006, Australia

Correspondence: k.etebari@uq.edu.au

PIWI interacting RNAs (piRNA) have been shown to play important roles at the forefront of defence against transposable elements (TE) in germline cells. Their expression in other tissues is an

indicator of other possible functionalities for this group of non-coding RNAs. Here we report the piRNA profile of the dengue mosquito, *Aedes aegypti*, and their differential expression patterns in response to microbial challenges and a xenobiotic stress. Our deep sequencing data showed that a low proportion of host endogenous piRNAs (7%) are transposon derived and the majority of these TE linked piRNAs mapped to LTR retrotransposons. Dengue virus and the endosymbiotic bacterium, *Wolbachia*, infection significantly reduced piRNA abundance in adult mosquitoes and the Aag2 cell line derived from the mosquito. In contrast, the majority of differentially expressed piRNAs showed significant up-regulation after exposure to the sub-lethal dose of a pyrethroid insecticide as a chemical stress inducer. Although insecticide exposure, arbovirus and *Wolbachia* infection possibly trigger different pathways in the host, we found 100 differentially expressed piRNAs common among the datasets from these three treatments. These findings further indicate that piRNAs may play significant roles not only in TE silencing activity but also in host-pathogen interaction and stress responses.

CONTRIBUTED PAPER Monday 17:00 **32-STU**

A modified viral shuttle for exploring RNAi-related genes

David Neunemann¹, Heiko Vogel¹, David G. Heckel¹, Salvador Herrero², Ágata K. Jakubowska²

¹Entomology Department, Max Planck Institute for Chemical Ecology, Jena, Germany; ²Department of Genetics, Universitat de València, Burjassot, Spain

Correspondence: dneunemann@ice.mpg.de

Gene silencing via dsRNA has become a powerful tool to explore functional genomics in a wide variety of eukaryotic organisms. However, RNA interference (RNAi) is still in the exploratory phase in non-model organisms. Especially in Lepidoptera, RNAi is not as straight-forward and efficient as in other insects and it has been proven to be difficult to establish, exemplified by a larger number of studies done in the past years in different Lepidopteran species with sometimes questionable effects. We analyzed RNAi-related gene expression levels (miRNA-pathway and siRNA-pathway) in *Helicoverpa armigera* and *Heliothis virescens* 5th instar larva in different tissues. We found that R2D2 is transcribed at very low levels in all tissues except testes, whereas Loquacious is transcribed at very high levels in all tissues. These results could suggest that, despite appropriate design, the dsRNA failed to enter the siRNA pathway and knock-down the gene of interest due to the observed very low levels of R2D2. As the siRNA pathway is also known as the "antiviral pathway" and defends the organism against RNA and DNA viruses, we analyzed RNAi related genes after infection of *Helicoverpa armigera* with an *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) and *Helicoverpa armigera* single nucleopolyhedrovirus (HaSNPV). After 48 h infection with AcMNPV we could observe an induction of R2D2. Based on these results we will now test the possibility of using a modified AcMNPV, which lacks an important transcription factor and carries a dsRNA construct, as a shuttle to deliver dsRNA into cells, potentially resulting in target gene knockdown.

CONTRIBUTED PAPER Monday 17:15 **33-STU**

Role of dsRNA-mediated mechanism in the establishment of "latent" *Glossina hytrosavirus* infections in the tsetse fly

Irene K. Meki^{1,2}, Henry M. Kariithi³, Just M. Vlak¹, Adly M. M. Abd-Alla², Monique M. van Oers¹

¹Laboratory of Virology, Wageningen University, The Netherlands;

²Insect Pest Control Laboratory, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, Vienna, Austria;

³Biotechnology Research Institute, Kenya Agricultural & Livestock Research Organization, Kaptagat Rd, Loresho, Nairobi, Kenya
Correspondence: a.m.abd-alla@iaea.org,
monique.vanoers@wur.nl

Tsetse flies (Diptera; Glossinidae) are naturally infected by *Glossina* hytrosavirus (family Hytrosaviridae), a large dsDNA virus pathogenic specifically to the *Glossina* spp. The virus infections are largely asymptomatic. Whereas *G. pallidipes* is more susceptible to the virus infection, infection in *G. m. morsitans* is predominantly asymptomatic. In *G. pallidipes*, asymptomatic infection can convert to symptomatic infection that is characterized by salivary gland hypertrophy syndrome (SGH), leading to reproductive dysfunction of infected flies. It is hypothesized that the virus infection is maintained by dsRNA-mediated gene silencing, such that only a few of the viral genes are expressed during asymptomatic infections to avoid detection by the host's immune system. To investigate whether host-mediated dsRNA mechanism(s) is involved in maintenance of asymptomatic virus infection, we performed a comparative analysis of expression levels of *Dcr-2* and *Ago-2* genes in *G. pallidipes* and *G. m. morsitans*. Whereas *Ago-2* and *Dcr-2* were significantly up- and down-regulated in symptomatic and asymptomatic *G. pallidipes*, respectively, the two genes were down- and up-regulated in infected and uninfected *G. m. morsitans*, respectively. Further, transcriptomic data indicated that miRNAs may be involved in the conversion of asymptomatic to the symptomatic infection states. These findings implicate dsRNA gene silencing (perhaps through miRNAs) is involved in controlling the virus infection in tsetse flies. These data provide avenues for further investigations into the roles of RNAi during latent hytrosavirus infections in *Glossina*.

CONTRIBUTED PAPER Monday 17:30 **34-STU**

New target sites in pest control: Examining the role of REPAT proteins in defence against baculoviruses

Esençül Özdemir¹, *Umut Toprak¹*, *Sibel Çavdar¹*, *Diana Bekkaoui²*,
Dwayne Hegedus²

¹Ankara University, Faculty of Agriculture, Dept. of Plant Protection, Ankara, Turkey; ²Agriculture and Agri-Food Canada, Saskatoon Research Centre, Saskatoon, SK Canada

Correspondence: utoprak@agri.ankara.edu.tr

Baculoviruses are invertebrate-specific pathogens with great potential for being developed as biopesticides; however, their slow speed of killing has been a barrier for widespread use. Development of more effective baculoviruses requires a detailed understanding of their infection mechanism. Baculoviruses initiate infection from midgut epithelial cells. This requires passage through a porous structure lining the midgut, the peritrophic matrix, which is composed of chitin and multiple proteins. One group of proteins associated with the PM are from the REPAT (Response to pathogen) protein family that have been shown to be involved in the defense against pathogens. The objective of this study was to identify the REPAT proteins in *Spodoptera littoralis* (Lepidoptera:Noctuidae), a major pest of cotton and vegetables in North Africa, Mediterranean region and the Middle-East. Scanning of a *S. littoralis* midgut-specific cDNA library revealed 11 REPAT (SpliREPAT1-11) proteins. Full length cDNAs were obtained by RACE analyses. Site-specific gene expression analyses by droplet digital PCR indicated that they were predominantly expressed in the midgut, while developmental gene expression analyses indicated that expression was specific to larval stages. Inoculation of 3rd instar larvae by a baculovirus revealed an upregulation in the expression of REPAT genes, suggesting they have a defense-related role. In

conclusion, targeting defense-related midgut genes encoding proteins such as REPATs could help development of improved baculoviruses in pest control strategies.

CONTRIBUTED PAPER Monday 17:45 **35-STU**

***Wolbachia*-mediated antiviral protection is life stage dependent**

Aleksej L. Stevanovic, *Pieter A. Arnold*, *Karyn N. Johnson*,
School of Biological Sciences, The University of Queensland,
Brisbane 4072, Australia

Correspondence: aleksej.stevanovic@uqconnect.edu.au

Viral infection and maintenance within insect populations is dependant on the pool of virus present within the system. The endosymbiotic bacterium *Wolbachia* has the ability to limit viral replication in adult *Drosophila* flies and mosquitoes, a feature that can be utilised to limit virus spread. To understand the effect of *Wolbachia* on insect populations, it is important to consider viral susceptibility throughout all developmental stages. We used *Wolbachia*-host combinations known to exhibit antiviral protection in adult flies, and investigated protection in larvae. *Drosophila* C virus was delivered to first instar larvae through oral feeding, and survival was scored to determine if antiviral protection was retained at larval stages. *Wolbachia* mediated protection was lost in all but one *Wolbachia*-host combination, wMelPop in W1118. Because *Wolbachia* density has been previously shown to be important in conferring antiviral protection in adult flies, we investigated *Wolbachia* density at the larval stages using RT-qPCR. The expression analysis showed that 5 out of 6 *Wolbachia* strains examined show reduced *Wolbachia* levels at larval compared to adult stages. Interestingly, the wMelPop strain, which retained high *Wolbachia* densities in larvae, was the only strain that provided protection at both the larval and adult stages, indicating that *Wolbachia* density is important for antiviral protection throughout all stages of development. Taken together these data show that *Wolbachia*-mediated antiviral protection can vary between life stages of an insect. We therefore suggest that consideration be given in selecting *Wolbachia* strains that confer protection at all stages of development when designing programs focused on minimizing the spread of insect vectored viruses.

CONTRIBUTED PAPER Monday 18:00 **36-STU**

A viral histone H4 modulates host gene expression by down regulating chromatin remodeling machinery

Sunil Kumar, *Yonggyun Kim*

Department of Bioresource Sciences, Andong National University,
Andong 760-749, Republic of Korea

Correspondence: sunilkumar_micro@rediffmail.com

Histone H4 is a protein subunit of nucleosomes in eukaryotes and play crucial roles in DNA packaging, chromatin formation and in regulation of gene expression by covalent modification. A viral histone H4 is encoded in a polydnavirus called *Cotesia plutellae* bracovirus (CpBV). The viral H4 (CpBV-H4) is highly homologous with other H4 proteins except 38 extended residues in N terminus. Its expression alters insect gene expression and suppresses immune and development. In this study, CpBV-H4 was expressed in a natural host, *Plutella xylostella*, and its suppressive activity on host gene expression was detected by suppressive subtractive hybridization (SSH) technique. SSH targets, of which expressions were down-regulated by CpBV-H4, were read by 454 pyrosequencing and annotated using the published *P. xylostella* whole genome. Resulting targets were assigned to most GO functional categories. Two chromatin remodeling factors were included in the SSH targets. Lysine demethylase (Px-KDM) of *P.*

xylostella was highly expressed during entire larval period in all tested tissues. However, the suppression of Px-KDM expression by a specific RNA interference (RNAi) did not affect immune response, but significantly impaired the larval development. SWI/SNF of *P. xylostella* (Px-SWI/SNF) was expressed in all developmental stages. Its RNAi did not affect larval development, but led to significant alteration in adult metamorphosis. CpBV-H4 suppressed expressions of both Px-KDM and Px-SWI/SNF, but its truncated mutant lacking in the extended N-terminal tail did not. These results suggest that the biological alteration in *P. xylostella* parasitized by *C. plutellae* can be caused by an epigenetic control of CpBV-H4 against chromatin remodeling factors.

CONTRIBUTED PAPER Monday 18:15 **37-STU**

Infection studies of an *Agrotis segetum* granulovirus isolate from Europe in cell cultures of AiE1611T

*Gianpiero Gueli Alletti*¹, *Jörg T. Wennmann*¹, *Marina Eigenbrodt*¹, *Eric B. Carstens*^{1,2}, *Regina G. Kleespies*¹, *Johannes A. Jehle*¹

¹Julius Kühn Institute, Heinrichstr. 243, D-64287 Darmstadt, Germany; ²Queen's University, K7L 3N6 Kingston (ON), Canada

Correspondence: gianpiero.gueli.alletti@jki.bund.de

Larvae of *Agrotis segetum*, the common cutworm (Denis & Schiffermüller) and *A. ipsilon*, the black or greasy cutworm (Hufnagel) are polyphagous lepidopteran pests, which cause severe damage on roots and stems of numerous cultivated plants and are of significant economic importance. Four baculoviruses, namely *Agrotis segetum* nucleopolyhedrovirus A (AgseNPV-A), *Agrotis segetum* nucleopolyhedrovirus B (AgseNPV-B), *Agrotis ipsilon* nucleopolyhedrovirus (AgipNPV), and *Agrotis segetum* granulovirus (AgseGV), belonging to the genera *Alpha*- and *Betabaculovirus*, respectively, are known to infect larvae of *A. segetum* and *A. ipsilon*. Infections with these baculoviruses were characterized in dose-response bioassays and with molecular biological methods in neonate *A. segetum* larvae. Mixed infection of single *A. segetum* larvae with AgseNPV and AgseGV have been reported. As a further step to improve the understanding of the biology of AgseNPV-B and AgseGV in single and mixed infections, plaque purified virus stocks of these two viruses were prepared in the *A. ipsilon* cell line AiE1611T. Infected cell samples were characterized with light/electron microscopy and PCR assays. Genomic stability of AgseNPV-B and AgseGV were investigated in RFLP and sequence analysis. These analyses comprised genomic comparisons between two AgseGV isolates from China (AgseGV-XJ, AgseGV-L1) and the isolate in this study, AgseGV-JKI. These investigations revealed the ability of both AgseNPV-B and AgseGV to replicate in AiE1611T.

CONTRIBUTED PAPERS Monday 16:30 – 17:30

Microsporidia

CONTRIBUTED PAPER Monday 16:30 **38**

Host-pathogen interactions and genome evolution in two generalist and specialist microsporidian pathogens of mosquitoes

*James J. Becnel*¹, *Christopher A. Desjardins*², *Neil Sanscrainte*¹, *Jonathan M. Goldberg*², *David Heiman*², *Sarah Young*², *Qiandong Zeng*², *Hiten D. Madhani*³, *Christina A. Cuomo*²

¹USDA, ARS, Center for Medical, Agricultural and Veterinary Entomology, 1600 SW 23rd Drive, Gainesville, Florida 32608, USA;

²Broad Institute of MIT and Harvard, Cambridge, Massachusetts 02142, USA; ³Department of Biochemistry and Biophysics,

University of California-San Francisco, San Francisco, California 94158, USA

Correspondence: James.Becnel@ars.usda.gov

The adaptation of two distantly related microsporidia to their mosquito hosts was investigated. *Edhazardia aedis* is a specialist pathogen that infects *Aedes aegypti*, the main vector of dengue and yellow fever arboviruses. *Vavraia culicis floridensis* is a generalist pathogen of several insects including *Anopheles* spp. that vector malaria. Genomic analysis and deep RNA-Seq across infection timecourses reveal fundamental differences between these two pathogens. *E. aedis* retains enhanced cell surface modification and signaling capacity, upregulating protein trafficking and secretion dynamically during infection. *V. culicis* is less dependent on its host for basic metabolites and retains a subset of spliceosomal components, with a transcriptome broadly focused on growth and replication. Transcriptional profiling of mosquito immune responses reveals that response to infection by *E. aedis* differs dramatically depending on the mode of infection, and that antimicrobial defensins may play a general role in mosquito defense against Microsporidia. This analysis illuminates fundamentally different evolutionary paths and host interplay of specialist and generalist pathogens of microsporidia.

CONTRIBUTED PAPER Monday 16:45 **39**

Ultrastructural analysis and SSU rRNA gene sequencing of *Alfvenia* sp. and *Agglomerata cladocera* from Siberian microcrustaceans shed light on diversification within the "Aquatic Outgroup"

Yuliya Y. Sokolova^{1,3}, *Yuri S. Tokarev*², *Georgiy I. Rusakovich*², *Igor V. Senderskiy*²

¹Institute of Cytology; ²Institute for Plant Protection, Russian Academy of Sciences, St. Petersburg, Russia; ³School of Veterinary Medicine, Louisiana State University, Baton Rouge LA, USA

Correspondence: sokolova@lsu.edu

Two microsporidia from freshwater crustaceans were discovered during the ongoing survey for microsporidia in the river Karasuk basin (Novosibirsk Region, Western Siberia). The first species, tentatively a new species of the genus *Alfvenia*, parasitizes *Cyclops* sp. (Maxillopoda, Copepoda). The second one infects a cladoceran *Daphnia magna* (Branchiopoda, Phyllophoda), and basing on ultrastructure, was identified as *Agglomerata (Glugea) cladocera* (Pfeifer, 1895) Larsson, Dieter, Vavra, 1996, comb. n. Siberian isolate of *A. cladocera* shares 99% SSU rRNA gene sequence similarity to *Binucleata daphniae* Refardt et al., 2008 from Belgium. No sequences belonging to representatives of genera *Alfvenia* or *Agglomerata* have been available so far through public databases. New information sheds light on phylogenetic bonds within the Aquasporidia group of the Clade I (Vossbrinck, Debrunner-Vossbrinck, 2005). Both parasites fell in the "Aquatic outgroup" (AOG), a sister to the *Amblyospora* clade. *Agglomerata cladocera* clusters with *Binucleata daphniae*, while *Alfvenia* sp. – with *Trichoutzetia guttata*. Interestingly, two major well supported branches can be identified within AOG: members of the first, the *Alfvenia-Trichoutzetia* lineage parasitize predominantly copepods-cyclopids, and representatives of the second, the *Agglomerata-Gurleya-Larssonia-Berwaldia* lineage are mainly associated with cladocerans. AOG clade also includes a few taxa with dipteran hosts suggesting that common ancestors of amblyosporids and AOG possessed polyxenous life cycles that probably involved a copepod as an intermediate host, like some extant amblyosporids. Given morphological and sequence similarity of *Binucleata daphniae* to *Agglomerata* spp., the genus *Binucleata* may be amended to a junior synonym of *Agglomerata*. Supported by RFBR 13-04-00693 and RF President grant MD-4284.2015.4.

CONTRIBUTED PAPER Monday 17:00 **40-STU****Understanding the evolutionary loss of glycolysis in intranuclear crab microsporidians and the role of their highly mutated hexokinase in their unusual habitat***Dominic Wiredu Boakye¹, Bryony Williams¹, Grant Stentiford², Thomas Williams³*¹College of Life and Environmental Sciences, University of Exeter, Geoffrey Pope, Stocker Road, Exeter EX4 4QD; ²Centre of Environment, Fisheries and Aquaculture Science, CEFAS, The Nothe, Barrack Road, Weymouth, UK; ³Institute for Cell and Molecular Biosciences, University of Newcastle, Newcastle upon Tyne, Tyne and Wear, United Kingdom
Correspondence: dw347@exeter.ac.uk

Glycolysis is a metabolic process that breaks glucose into energy and is ubiquitous in eukaryotic cells. However *E. bienersi*, a human infecting microsporidian has been recently found to lack this pathway. Loss of this metabolic process in conjunction with the general absence of oxidative phosphorylation capabilities in the Microsporidia suggests that *E. bienersi* solely relies on ATP import for its energy needs.

The placement of *E. canceri*, a crab infecting intranuclear microsporidia as the closest relative to *E. bienersi* in phylogenetic studies sparked questions of whether this crab parasite has also lost glycolytic capabilities and how it attained energy from within its unusual intranuclear habitat. Comparative genomic analyses in this study have partly answered this question by confirming the absence of most glycolytic genes from not only the genome of *E. canceri* but from other members of the Enterocytozoon family as well. However hexokinase, the gene that codes for the first protein of the glycolytic pathway appears to be retained in the genome of all members of the Enterocytozoon family.

Further comparative analysis has also revealed that the catalytic domains of this gene have undergone severe deletion mutations and in the case of *E. canceri*, an entire new domain is adjoined to the N-terminal of the predicted protein. Here, we are using kinetic and high-throughput phenotype assays to test the functionality and substrate specificity of these microsporidian hexokinases in order to better understand the role of these mutated proteins in microsporidian carbon metabolism and intranuclear living.

CONTRIBUTED PAPER Monday 17:15 **41-STU****Characterization of the spliceosome and large introns in microsporidia***Thomas A. Whelan, Cameron J. Grisdale, Naomi M. Fast*

Department of Botany, University of British Columbia, Vancouver, BC, Canada

Correspondence: whelant@alumni.ubc.ca

Microsporidia are fungal intracellular parasites that have some of the most reduced and compacted genomes known. This reduction is particularly evident when looking at the spliceosome and spliceosomal introns. The genome of *Encephalitozoon cuniculi* is only 2.9 Mb, has lost the majority of its introns, and we predict its reduced spliceosome lacks the U1 snRNA, a component of the spliceosome that identifies introns early in the splicing process. Only 37 introns remain and they are predominantly shorter than 25 nt. To assess whether the dramatic reduction in intron length and spliceosome size affects the splicing process, we carried out high-throughput transcriptome sequencing to determine splicing levels. Our analysis showed that the majority of introns in *E. cuniculi* were spliced at levels much lower than seen previously in other reduced fungi. One intron in the Poly-A ORF stood out not only because of its size (76 nt) but it was also spliced in 80% of the transcriptome reads. Of interest, two similarly large introns were

independently found in *Spraguea lophii*: one in the Poly-A ORF (as in *E. cuniculi*) and one in the S23 ribosomal protein gene. We have now identified additional long introns with unusual levels of sequence conservation. We found that 14 of 20 microsporidian genomes possess a Poly-A intron and 6 have an S23 intron. Assessing the distribution of these introns could provide us with insights into intron gain, a process rarely observed, and could also highlight a potential regulatory role for splicing in microsporidia.

TUESDAY – August 11th

SYMPOSIUM BACTERIA Tuesday 8:00 – 10:00

Mechanisms of Field Resistance to Bt Pesticides and Bt-CropsSYMPOSIUM PAPER Tuesday 8:00 **42****Bt resistance in *Plutella* – too many trees?***Neil Crickmore*

School of Life Sciences, University of Sussex, Brighton, UK

Correspondence: n.crickmore@sussex.ac.uk

Plutella xylostella was the first pest insect in which field resistance to Bt was reported and consequently much work has been done in an attempt to understand the underlying mechanism. Most resistant populations conform to the so-called Mode 1 phenotype in which resistance to Cry1A toxins is observed but little cross-resistance to Cry1C exists, resistance is recessive and characterized by the loss of binding of the Cry1A toxin(s). The similarity in these phenotypes, along with complementation data, suggested a commonality between the different resistant populations, yet detailed studies suggested a much more complex picture. Various reports for example have suggested that the following may all contribute to the resistance phenotype: multiple alleles, complex toxin binding scenarios, cross-resistance with pyrethroids, involvement of esterases, reduced proteolytic processing, altered membrane composition and the involvement of gut microbiota. More recent work has mapped the resistance allele to the ABCC2 locus in at least two resistant populations and “omics” studies, combined with RNAi, have identified a number of genes that appear to contribute to the resistance phenotype. In this presentation I will consider to what extent the role of many of the above factors in resistance is justified by the experimental data and whether too much data can mask a more straightforward explanation. Finally I will consider whether there might be a unifying hypothesis, based on a single genetic lesion, that can explain the various results obtained. Although the presentation will use *Plutella* as an example the analysis could be applicable to other pest species.

SYMPOSIUM PAPER Tuesday 8:22 **43****Multiple resistance mechanisms selected in cabbage looper populations resistant to DiPel***Ping Wang*Department of Entomology, Cornell University
New York State Agricultural Experiment Station, Geneva, NY
14456Correspondence: pingwang@cornell.edu

The cabbage looper, *Trichoplusia ni*, is one of only two insect species that have developed resistance to Bt sprays in an

agricultural environment. The GLEN population of *T. ni* developed resistance to DiPel, a formulation of *Bacillus thuringiensis* var. *kurstaki* (Btk), in commercial greenhouses (Janmaat and Myers, 2003. *Proc. Biol. Sci.*, 270: 2263-2279). The DiPel-resistant GLEN strain, GLEN-DiPel, is highly resistant to Cry1Ac, a major Cry toxin in Btk. In the GLEN-DiPel population, resistance to Cry2Ab was also selected. Cry1Ac-resistance and Cry2Ab-resistance are conferred by two genetically independent mechanisms. The Cry1Ac resistance is associated with down-regulation of APN1 gene expression, and the resistance is controlled by a *trans*-acting resistance gene localized in the ABC transporter ABCC2 gene locus region. Cry2Ab resistance is conferred by a novel yet to be understood genetic mechanism. The presence of both Cry1Ac resistance and Cry2Ab resistance mechanisms in *T. ni* lead to resistance of *T. ni* larvae to the dual toxin Bt-cotton variety BollGard II.

SYMPOSIUM PAPER Tuesday 8:44 **44**

Pink bollworm resistance to Bt cotton: Similar mechanism in the lab and field?

*Jeffrey A. Fabrick*¹, *Jeyakumar Ponnuraj*², *Xianchun Li*³, *Yves Carrière*³, *Bruce E. Tabashnik*³

¹USDA-ARS, U.S. Arid Land Agricultural Research Center, Maricopa, Arizona, USA; ²National Institute of Plant Health Management, Rajendranagar, Hyderabad, Andhra Pradesh, India; ³Department of Entomology, University of Arizona, Tucson, Arizona, USA

Correspondence: jeff.fabrick@ars.usda.gov

Evolution of resistance by insect pests can reduce the benefits of insecticidal proteins from *Bacillus thuringiensis* (Bt) that are used extensively in sprays and transgenic crops. Despite considerable knowledge of the genes conferring insect resistance to Bt toxins in laboratory-selected strains and in field populations exposed to Bt sprays, understanding of the genetic basis of field-evolved resistance to Bt crops remains limited. We found that in pink bollworm (*Pectinophora gossypiella*), both laboratory-selected resistance to Cry1Ac and field-evolved resistance to Cry1Ac in Bt cotton are associated with mutations in a gene encoding a cadherin protein that binds Cry1Ac. Analysis of thousands of individuals from five laboratory-selected strains from the United States revealed four cadherin alleles that are genetically linked with resistance to Cry1Ac. None of these four cadherin resistance alleles from the US strains were detected in screening DNA from 436 individuals collected from seven field populations in India. However, in only seven individuals collected from two field populations in India, we found eight novel, severely disrupted cadherin alleles associated with field-evolved resistance to Cry1Ac. For these eight alleles from India, analysis of complementary DNA (cDNA) revealed 19 transcript isoforms, each containing a premature stop codon, a deletion of at least 99 base pairs, or both. Seven of the eight disrupted alleles each produced two or more different transcript isoforms, which implicates alternative splicing of messenger RNA (mRNA). This represents the first example of alternative splicing associated with field-evolved resistance that reduced the efficacy of a Bt crop.

SYMPOSIUM PAPER Tuesday 9:06 **45**

Mechanism of *Spodoptera frugiperda* resistance to Cry1Fa in Bt corn

*Juan Luis Jurat-Fuentes*¹, *Siva R. K. Jakka*¹, *Rahul Banerje*¹, *James Hasler*², *Fangneng Huang*³, *Robert Meagher*⁴, *Rodney Nagoshi*⁴

¹Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN, USA; ²DOW AgroSciences, Indianapolis, IN, USA; ³Department of Entomology, Louisiana State University

AgCenter, Baton Rouge, LA, USA; ⁴USDA-ARS, Insect Behavior and Biocontrol Research Unit, Gainesville, FL, USA

Correspondence: jurat@utk.edu

Field-evolved resistance to transgenic corn producing the Cry1Fa insecticidal protein from *Bacillus thuringiensis* was detected and reported in populations of *Spodoptera frugiperda* (fall armyworm) from Puerto Rico in 2006. Resistance resulted in relevant field control failures, leading to voluntary withdrawal of the corn event from the local market. Using field-collected insects we have studied resistance genetics and fitness costs, as well as the mechanisms responsible for resistance. We have found that resistance to Bt corn in *S. frugiperda* is autosomally inherited and highly recessive, yet we failed to detect relevant fitness costs when compared to a susceptible colony. The Cry1Fa-resistant *S. frugiperda* were also cross-resistant to Cry1A insecticidal proteins, but not to the Cry1Ca protein. Comparison of Cry protein binding to brush border membrane vesicles prepared from susceptible and resistant *S. frugiperda* revealed a dramatic reduction of Cry1A and Cry1Fa toxin binding in resistant larvae. In contrast, Cry1Ca binding was not different among the compared strains, supporting an association between reduced binding and resistance and cross-resistance phenotypes. Considering this association, we examined alterations in putative Cry1 protein receptors in resistant *S. frugiperda*. Real time-PCR analyses detected a dramatic down-regulation of specific alkaline phosphatase isoforms in larvae resistant to Cry1Fa corn. In this presentation we will share and discuss recent data towards the mechanistic description of resistance to Cry1Fa corn in *S. frugiperda*.

SYMPOSIUM PAPER Tuesday 9:28 **46**

Characterization of Cry3Bb1 resistance in western corn rootworm (*Diabrotica virgifera virgifera*)

Jeffrey A. Haas

Monsanto Company, 700 Chesterfield Parkway West, Chesterfield, MO 63017, USA

Correspondence: jeff.a.haas@monsanto.com

Western corn rootworm (WCR) is a significant pest of corn causing >\$1B US in crop damage annually. Observations of WCR resistance to corn hybrids expressing the *Bacillus thuringiensis* 3-domain Cry protein (Bt; 3D-Cry), Cry3Bb1, have been documented, particularly in areas of repeated corn-on-corn production where hybrids expressing a single mode of action were grown (Gassmann, 2011). More recently, evidence for cross-resistance to both Cry3Bb1 and to mCry3Aa has been published (Gassmann, 2014). Previous biochemical studies of a WCR population derived from Hopkinton, IA, and reared under continuous MON88017 selection, did not reveal a clear mechanism for Cry3Bb1 resistance. While *in vitro* binding and proteolytic processing appeared unchanged, RNASeq and proteomic analyses of whole larvae revealed changes in immune/pathogen response genes. However, involvement of these genes in Cry3Bb1 resistance could not be validated by RNAi feeding studies in WCR. Putative Cry1 receptor homologs (ex. ABC transporters, cadherin, alkaline phosphatase, aminopeptidase) were not detected in whole larval proteomics and RNA expression profiles were not significantly different when whole larvae of susceptible and resistant colonies were compared. Recently, detection of potential 3D-Cry receptor families has been observed by proteomic analysis of brush border membranes isolated from WCR larvae. Results from a comparative analysis of Cry3Bb1 susceptible and resistant WCR colonies will be discussed in the context of previous biochemical,

molecular, and genetic observations, and in relation to potential resistance mechanisms in WCR.

CONTRIBUTED PAPERS Tuesday 8:30 – 9:45

Diseases of Beneficial Invertebrates 1

CONTRIBUTED PAPER Tuesday 8:30 **47**

Pathogens on the horizon: Enhancing understanding of invasive alien entomopathogens and impacts on biodiversity

Helen Hesketh¹, Jørgen Eilenberg², Monique van Oers³, Helen Roy¹, Alison Dunn⁴, Grant Stentiford⁵, Beth Purse¹, Regina Kleespies⁶, Anja Amtoft-Wyns²

¹Centre for Ecology & Hydrology (CEH), Wallingford, United Kingdom; ²University of Copenhagen, Denmark; ³Wageningen University, Netherlands; ⁴University of Leeds, United Kingdom; ⁵Centre for Environment, Fisheries and Aquaculture Science (Cefas), United Kingdom; ⁶Julius Kühn-Institut, Federal Research Institute for Cultivated Plants, Germany
Correspondence: hhesketh@ceh.ac.uk

Horizon-scanning, the systematic examination of future potential threats and opportunities, leading to prioritisation of invasive alien species (IAS) threats is an essential component of IAS management. Consensus methods have been used to undertake IAS horizon scanning for Great Britain; 30 species considered a high risk with respect to arriving, establishing and posing a threat to native biodiversity were identified. Information on microbes is often lacking from alien species databases and so pathogens were excluded from this consensus approach. There is a significant gap in our knowledge in relation to risk posed to biodiversity by “alien” microorganisms. We organised a workshop of 40 experts with five subgroups (terrestrial invertebrates, aquatic, plants, wildlife diseases and vectors of disease) to specifically consider invasive alien pathogens that have the potential to impact on biodiversity in the EU. The aim was to undertake a cross-cutting review of pathogen life histories to enhance understanding of threats, methods and knowledge gaps within natural and semi-natural systems, and to identify invasive alien pathogens that will impact on biodiversity in Europe. We identified key barriers to our understanding of the introduction, establishment and impact of invasive alien pathogens through two distinct phases; 1) preliminary consultation between experts prior to the workshop, 2) consensus-building across the expert groups during the workshop. We highlight the results of this consensus approach by examining the top 10 barriers identified. We then detail specific examples from the terrestrial invertebrate and aquatic sub-groups that exemplify these barriers and indicate how this may inform pan-European policy.

CONTRIBUTED PAPER Tuesday 8:45 **49**

The prevalence of multi-host pathogens in *Bombus pascuorum* is influenced by honeybee domestication

Ivan Meeus¹, Laurian Parmentier¹, Jafar Maharramov¹, Niels Piot¹, Dirk C. de Graaf², Guy Smagghe¹

¹Department of Crop Protection, Ghent University, Coupure links 653, B-9000 Ghent, Belgium; ²Department of Biochemistry and microbiology, Ghent University, Ghent, Belgium
Correspondence: ivan.meeus@UGent.be

Domesticated animals are known as potential sources of parasites and viruses spilling over toward sympatric wild life. Their relatedness with the wild species and overlap in habitat are two important parameters for pathogen spillover. We question

whether the domesticated honeybee could act as a reservoir of parasites and viruses, facilitating the spillover toward wild bumblebees. As spillover of pathogens is one of the main drivers of emergent infectious diseases and could represent an important factor in the multi-factorial problem of bumblebee decline. We followed parasite (n = 3) and virus (n = 4) prevalence in *Bombus pascuorum* (n = 100) in five locations, each location having a study site rich with honeybees, being close to apiaries, paired with a study site with low honeybee counts. Study sites more distant from apiaries had decreased *Apicystis bombi* and virus infection, specifically sacbrood virus (SBV) and viruses from the AKI complex (acute bee paralysis virus, Kashmir bee virus and Israeli acute paralysis virus complex). We conclude that presence of domesticated honeybees can interfere with wild host-pathogen interactions. This disturbance can occur because the honeybees are infected by the pathogens and/or because they are the most abundant pollinator in our setup and could spread the pathogens by external vectoring. We discuss possible mitigation actions for the beekeeping sector, basically advising the reduction of 1) the pathogen load of bee hives, 2) the sector’s mobility and 3) bee hive abundance, especially in vulnerable ecosystems.

CONTRIBUTED PAPER Tuesday 9:00 **50-STU**

From the mosquito model to the bumblebee: A different behaviour of *Vago* mediated cross-talk between the small interfering RNA and Jak/stat pathways upon virus infection

Jinzhi Niu, Ivan Meeus, Guy Smagghe

Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, Coupure links 653, B-9000 Ghent, Belgium
Correspondence: Jinzi.niu@ugent.be

Both small interfering RNA (siRNA) and Jak/stat pathways are recognized as important host defense mechanisms against virus infection. In mosquito, they are not only activated during virus infection, but also present a cross-talk through *Vago* in a *Dicer-2* dependent manner. Upon virus infection, the upregulation of *Dicer-2* leads to the activation of *Vago* transcription, which increases the level of secreted *Vago*. Subsequently, *Vago* influences Jak/stat signaling. In current study, we comparatively analyzed the bumblebee siRNA response to systemic infection of virulent -Israeli acute paralysis virus (IAPV) and avirulent -slow bee paralysis virus. Both virus infections can induce the expression of *Dicer-2*, which was also confirmed by production of virus derived siRNAs during IAPV infection. A “RNAi of RNAi” strategy was used to pre-silencing *Dicer-2*, which could impair the gene silencing efficiency, but it did not influence viruses genome copy number (gcn). With the upregulation of *Dicer-2* under IAPV infections, however, we observed the downregulation of *Vago*. The further cross-talk of *Vago* with Hop, the Janus kinase of the Jak/stat pathway, might be presented in bumblebees as silencing of *Vago* could downregulate the expression of *Hop*. In addition, the silencing of *Hop* in bumblebees leads to the increase of SBPV genome copy number (gcn). In order to further detect the effect of downregulation of *Vago* during IAPV infection, we tested the influences of pre-silencing *Vago* to virus infections. In summary, our results show a different behaviour of *Vago* upon virus infections in bumblebees, compared with the well-studied model in mosquito.

CONTRIBUTED PAPER Tuesday 9:15 **51**

Variations in disease profile of juvenile edible crabs (*Cancer pagurus*) sampled from 3 different locations in the UK

Kelly S. Bateman¹, Stuart Ross¹, Rose Kerr¹, Ruth Hicks², Nick Taylor², Grant D. Stentiford¹

¹European Union Reference Laboratory for Crustacean Diseases, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Barrack Road, The Nothe, Weymouth, Dorset DT4 8UB, UK; ²Centre for Environment, Fisheries and Aquaculture Science (Cefas), Barrack Road, The Nothe, , Dorset DT4 8UB, UK
Email: kelly.bateman@cefasc.co.uk

The edible crab (*Cancer pagurus*) is a major commercial species within Europe, total fishery production of edible crabs from European waters in 2013 was 49,263 metric tonnes, and 58% of this was landed in UK (www.fao.org/figis). Despite their commercial value relatively little is known about their susceptibility to disease and the effects that these diseases may have upon populations. It is known from previous research that the disease prevalence present in juvenile (un-fished) and adult (fished) populations differs, for example juvenile edible crabs have been shown to display different pathogen profiles when compared to adults of the same species from the same population (Bateman *et al.* 2011). We carried out a disease survey on juvenile crabs sampled from three distinct sites around the United Kingdom. Using histology, molecular biology and electron microscopy we surveyed 60 animals per month over a 12-month period. We show that the disease profiles varied between the three sites, pathogens prevalent at one site and absent in others, prevalence of others varying considerably between sites and month sampled. This study has highlighted the potential role of disease in leading to un-monitored (silent) mortalities in the unfished juvenile stocks, data will be incorporated into fisheries models to predict true risk of disease. This presentation will describe the pathologies and prevalence of these diseases during the 12 month study.

CONTRIBUTED PAPER Tuesday 9:30 **52**

Apicomplexan parasites cause regular mass mortalities in commercial scallop populations

Mark A. Freeman^{1,2}, Susan Inglis³, *Árni Kristmundsson*¹

¹Institute for Experimental Pathology at Keldur, University of Iceland, Reykjavik, Iceland; ²Institute of Ocean and Earth Sciences, University of Malaya, Kuala Lumpur, Malaysia; ³University of Massachusetts-Dartmouth, SMAST, Fairhaven, MA USA
Correspondence: mf33bitw@gmail.com

Scallops with reduced, discoloured and loosely bound adductor muscles have been reported from numerous scallop species from different locations in the North Atlantic. In most cases this condition has been associated with mass mortality events.

To determine the cause, four scallop species from different fisheries were examined. Iceland scallop, *Chlamys islandica* from Iceland; queen scallop, *Aequipecten opercularis*, from the Faroe Islands and Scotland, king scallops, *Pecten maximus* from Scotland and the sea scallop, *Placopecten magellanicus* from Georges Bank USA. Scallops were examined using wet mounts, histopathology, TEM and molecular methods.

Apicomplexan parasites were found in high numbers in all scallops examined. Healthy-looking scallops were lightly infected while scallops with clinical signs had severe infections. Infections were found in the adductor muscle and connective tissues of all organs causing extensive pathology. DNA sequencing revealed that the same parasite was responsible.

This apicomplexan has a wide distribution, is not specific with regards to scallop host and is highly pathogenic. Long-term studies in Icelandic waters, have shown that it is responsible for a total collapse of the scallop stock. Furthermore, it caused extensive pathology in sea scallops from Georges Bank and queen scallop in Faroese waters with both populations suffering abnormal mortality events. Dark and diminished adductor

muscles have also been reported from scallops from the Gulf of Alaska (*Patinopecten caurinus*) and the Barents Sea (*C. islandica*). Based on the clinical signs reported and the broad distribution of this apicomplexan, it seems probable that it is also the causative agent.

CONTRIBUTED PAPERS Tuesday 8:00 – 10:00

Viruses 2

CONTRIBUTED PAPER Tuesday 8:00 **53-STU**

AcMNPV encoded RING domain protein AC141 (Exon0) is associated with the GP64 and ME53 budding complex at the plasma membrane

*Siddhartha Biswas*¹, *Yang Liu*², *Peter J. Krell*², *David A. Theilmann*^{1,3}

¹Plant Science, Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC, Canada V6T 1Z4; ²Department of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario, Canada N1G 2W1; ³Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Summerland, British Columbia, Canada V0H 1Z0
Correspondence: siddharthbiswas.cu@gmail.com

During AcMNPV infection, nucleocapsids egress from the nucleus, traverse the cytoplasm and bud from the plasma membrane (PM) to form budded virus (BV). GP64 is an integral membrane protein that forms foci at the PM which are believed to be budding sites of nucleocapsids. ME53 is also required for efficient BV production and is found associated with GP64 foci and appears to form a budding complex. AC141 is a unique RING domain protein encoded by AcMNPV and deletion of this gene reduces the BV titer by 99%. Here we hypothesize that AC141 associates with the budding complex to facilitate the budding of nucleocapsids. Association of AC141 with ME53 and GP64 was analyzed by co-immunoprecipitation (Co-IP) and confocal microscopy in two different hosts, Sf9 and Tn5B1 cells, at 24 hpi. Cells were infected with a virus expressing ME53 tagged at the C-terminus with the HA epitope (ME53-HA). For confocal microscopy analysis a virus expressing ME53 fused at the C-terminal to EGFP (ME53:GFP) virus was used. Co-IP was performed with anti-HA agarose beads and eluent showed specific association of ME53 with AC141 as well as with GP64 and VP39. Reciprocal co-IP with AC141 polyclonal antisera also showed specific association with ME53, GP64 and VP39. These results suggested that AC141 was part of the budding complex. Further analysis using confocal microscopy examined co-localization of AC141, ME53:GFP and GP64. Distinct patterns of localization with GP64 foci were observed. Both AC141 and ME53 have nuclear and cytoplasmic localizations. AC141 localized adjacent to where ME53:GFP and GP64 co-localized at the budding complex.

CONTRIBUTED PAPER Tuesday 8:15 **54**

AcMNPV LEF-3 plays a critical role in both viral DNA replication and late gene expression

Mustapha El-Ayoubi, *Eric B. Carstens*

Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada
Correspondence: carstens@queensu.ca

Baculoviruses tightly coordinate different stages of viral gene expression with the initiation of viral DNA replication as a key process separating early and late phases. Early genes are transcribed by the host RNA polymerase. Late genes are

transcribed by a baculovirus-encoded RNA polymerase, composed of four subunits: LEF-4, LEF-8, LEF-9 and P47. We have determined the interactions of these subunits using fluorescent protein complementation assays. Each of the four subunits form homo-oligomers, when expressed on their own, and remain in the cytoplasm. All of the subunits form hetero-oligomers, except LEF-4 and LEF-8, which do not interact with each other. When tagged proteins were expressed in recombinant viruses, all the subunits interacted and localized to the nucleus. We also studied the interaction of these proteins with viral DNA replication genes including P143 and LEF-3. We previously showed that LEF-3 is responsible for transporting P143 into the nucleus. We have now discovered that the LEF-3 is also required for transporting the viral RNA polymerase subunits to the nucleus, and in the case of LEF-9, is essential for stabilizing the expressed protein. Additionally, we investigated the effect of temperature sensitive mutations in P47 and LEF-4 on their protein-protein interactions. The preliminary results demonstrated that single amino changes dramatically affected these interactions, and that LEF-3 was not able to overcome the defects. In summary, we will present data showing that the correct interaction of all the RNA polymerase subunits and their transport to the nucleus by LEF-3 are required for late viral gene expression.

CONTRIBUTED PAPER Tuesday 8:30 **55**

The ODV-E66 of *Helicoverpa armigera* nucleopolyhedrovirus is involved in viral oral infection but is not essential for BV synthesis

Dianhai Hou, Sijiani Luo, Fengqiao Zhou, Ranran Wang, Yanfang Zhang, Manli Wang, Hualin Wang, Zhihong Hu, Fei Deng
State Key laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, P.R. China
Correspondence: df@wh.iov.cn

Baculoviral ODV-E66 is a structural protein of the occlusion-derived virus (ODV) that has been revealed to be a novel viral chondroitinase. In this study, the function of *Helicoverpa armigera* nucleopolyhedrovirus (HearNPV) ODV-E66 was explored. *odv-e66* is a late gene, transcribed beginning at 18 h post infection until the very late stage of infection. *odv-e66* deleted- and repaired recombinant HearNPV viruses were subsequently generated and the one-step growth curve showed that deletion of *odv-e66* did not affect HearNPV BV replication *in vitro*. Electron microscopy showed that *odv-e66* deletion didn't cause an obvious change in ODVs/OBs morphology in comparison with the repaired virus. Bioassay results suggested that ODV-E66 facilitates the oral infection of HearNPV in *H. armigera* larvae. The LC₅₀ value of *odv-e66*-deleted virus against 3rd instar larvae increased ~3-fold, while this value increased ~30-fold for 4th instar larvae, compared with the repaired virus. It was previously postulated that the chondroitinase activity of ODV-E66 could partially digest the peritrophic membrane (PM) thus enhances *per os* infectivity. We proposed that the *odv-e66* deleted virus might be less capable to establish oral infection in elder-instar larvae because of the development of PM. Further in-depth investigations will be focused on the effect of *odv-e66* deletion on oral infectivity in larvae of different instars and the integrity of PM of larvae.

CONTRIBUTED PAPER Tuesday 8:45 **56**

Phosphorylation induces structural changes in the *Autographa californica* nucleopolyhedrovirus P10 protein

Linda A. King, Farheen Raza, Leo Graves, Sarah Irons, Robert D. Possee
Department of Biological and Medical Sciences, Oxford Brookes

University, Oxford OX3 0BP UK
Correspondence: laking@brookes.ac.uk

The baculovirus P10 protein is non-essential for virus replication in insect cells but has several roles very late in infection. It forms fibrillar structures in virus-infected cells that co-align with microtubules initially but later aggregate in the perinuclear region. P10 has been implicated in the application of the polyhedron envelope to occlusion bodies and also in nuclear lysis and release of polyhedra from virus-infected cells. Viruses lacking *p10* do not lyse insects as efficiently as the wild type but otherwise replicate normally. Phosphorylation of proteins can affect their structure and biological functions. The *Autographa californica* nucleopolyhedrovirus (AcMNPV) P10 has three potential phosphorylation sites at serine residues 84, 92 and 93. Using mutational analysis and MALDI-TOF mass spectrometry, we showed that P10 from AcMNPV-infected *Trichoplusia ni* TN368 cells at 72 hours post infection (hpi) was most likely phosphorylated at ser93. P10 phosphorylation mutants were expressed in TN368 cells and examined using confocal microscopy. This showed that mutation of ser93 caused conformational differences in P10 filament structures with delayed detachment from the nucleus very late in infection. The ser93 mutant appeared to cause normal nuclear lysis and release of occlusion bodies in TN368 cells. Interestingly, we noted that *Spodoptera frugiperda* (Sf21 and Sf9) did not undergo extensive nuclear lysis after infection with wild type AcMNPV as reported in previous studies, suggesting a degree of host specificity for P10 function. Further studies showed that AcMNPV P10 appears to act in concert with viral cathepsin but not chitinase to cause cellular and nuclear lysis.

CONTRIBUTED PAPER Tuesday 9:00 **57-STU**

***Bombyx mori* nucleopolyhedrovirus ARIF-1 enhances systemic infection in *B. mori* larvae**

Ryuhei Kokusho, Toru Shimada, Susumu Katsuma
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
Correspondence: katsuma@ss.ab.a.u-tokyo.ac.jp

Infection of lepidopteran-specific nucleopolyhedroviruses (NPVs) spreads systemically via hemolymph and tracheal systems. In each tissue, lepidopteran NPVs demonstrate a distinct tropism that results in diverse levels of viral propagation and gene expression according to the types of tissues. Although tissue tropism and the efficiency of systemic infection directly affect the final yield of progeny virus, the related factors and their detailed function are limitedly investigated. In this study, we identified a baculovirus gene, *actin rearrangement inducing factor-1 (arif-1)*, as a novel factor involved in systemic infection. *arif-1* was previously shown to be the inducer of filamentous actin concentration on the plasma membrane during the early stage of *Autographa californica* NPV infection in cultured cells. Our research revealed that *Bombyx mori* larvae infected with *arif-1*-deficient *B. mori* NPVs (BmNPVs) did not show locomotor hyperactivity that was normally observed in BmNPV-infected larvae. *arif-1*-deficient BmNPVs also showed reduced pathogenicity and total viral propagation in *B. mori* larvae, whereas viral propagation of *arif-1*-deficient viruses was comparable to control viruses in *B. mori* cultured cells. To monitor the spread of infection in tissues of *B. mori* larvae, an *arif-1*-defective BmNPV expressing *green fluorescent protein (gfp)* was injected into *B. mori* larvae. GFP expression and quantitative reverse transcription-polymerase chain reaction analyses revealed that the infection by the *arif-1*-disrupted virus was significantly delayed in trachea, fat body,

suboesophageal ganglion, and brain, but not in hemocytes. These results indicate that BmNPV ARIF-1 enhances systemic infection in *B. mori* larvae in a tissue-specific manner.

CONTRIBUTED PAPER Tuesday 9:15 **58**

Effect of me53 in baculovirus transcriptional regulation

*Yang Liu*¹, *Éva Nagy*², *Peter J. Krell*¹

¹Department of Molecular and Cellular Biology; ²Department of Pathobiology, University of Guelph, Guelph, ON, Canada

Correspondence: pkrell@uoguelph.ca

The conserved lepidopteran baculovirus protein ME53, is expressed both early and late during infection. The deletion of *me53* results in a significant decrease in virus production but no change in viral DNA synthesis. ME53 localizes to both the plasma membrane and nucleus of virus infected cells. As a nuclear protein with a conserved C4 zinc finger domain at its C-terminus which is usually considered to be related to transcriptional regulation, *me53* is consequently assumed to regulate transcription of viral genes. In order to determine if *me53* has an effect on transcriptional regulation, the transcript levels of 30 selected viral early and late genes were quantified by qRT-PCR in both wildtype bacmid transfected cells and *me53* knockout bacmid transfected cells. The presence of *me53* increases the transcription of some viral immediate early genes, genes encoding the viral RNA polymerase subunits, and some genes necessary for virus production. Moreover, the most highly up-regulated genes are the ones required for viral nucleocapsid assembly and egress. This suggests that *me53* might function as a transcription factor in the nucleus that regulates the expression of both viral early and late genes, in particular the late genes involved in virus production.

CONTRIBUTED PAPER Tuesday 9:30 **59**

Termination of invertebrate iridescent virus mRNA transcripts:

The role of a CATTa-containing hairpin

İkbal Agah İnce^{1,2}, *Monique M. van Oers*¹, *Gorben P. Pijlman*¹, *Just M. Vlask*²

¹Laboratory of Virology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands; ²Medical Microbiology Dept., Acibadem University Medical School, Istanbul, Turkey

Correspondence: monique.vanoers@wur.nl, agah.ince@acibadem.edu.tr

Invertebrate iridovirus mRNAs are not polyadenylated, in line with the absence of canonical polyA transcription termination motifs downstream of the open reading frames. Iridoviruses may apply a different termination method than most viral and host transcripts. Recently, we developed a method to determine the 3' ends of these non-polyadenylated mRNAs by adding a linker sequence to the 3' end using RNA-ligase. This linker can then be used to anneal to a specific primer to carry out RT-PCR. Using this method, we analyzed the transcripts of *Chilo iridescent virus* (CIV) or invertebrate iridescent virus 6 (IIV-6) upon infection of *Drosophila Schneider* 2 (S2) cells. The sequence of the 3' untranslated region of the cDNAs obtained was examined. Most transcripts terminated at or after a CATTa (CAUUA in RNA) sequence or a close variant thereof. The complementary motifs CATTa and TAATG were strongly enriched in the 100 basepairs downstream of CIV ORFs compared to their occurrence in the whole CIV genome. In the viral genome these motifs are present in larger inverted repeat sequences with the ability to form hairpin (stem-loop) structures varying in length between 22 and 56 nt. The *in silico* and *in vivo* analyses suggest that hairpin-based transcription termination is a conserved characteristic in the genus *Iridovirus*

(at least for IIV-6 and IIV-9), which may represent a novel transcript termination mechanism. The iridovirus situation may be comparable to the termination of metazoan histone transcripts, where a stem-loop structure controls mRNA termination.

CONTRIBUTED PAPER Tuesday 9:45 **60**

Post-translational modifications of the baculovirus protamine-like protein P6.9 and regulation of its hyperphosphorylation

Ao Li, *Haizhou Zhao*, *Qingying Lai*, *Zhihong Huang*, *Meijin Yuan*, *Kai Yang*

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

Correspondence: yangkai@mail.sysu.edu.cn

Diverse PTMs of histones and protamines constitute a code that creates highly selective binding platforms for the association of specific transcription regulatory proteins that determine the transcriptional state of genes. Many viral pathogens also utilize host- or virus-induced chromatin machinery to promote efficient infections. All of the insect-specific pathogen baculoviruses encode the protamine-like protein P6.9, which is required for versatile viral physiological processes. Currently, P6.9's post-translational modification sites and its regulating factors remain poorly understood. In the present study, we found that the P6.9 species could be categorized as unphosphorylated, hypophosphorylated, and hyperphosphorylated and that a virally encoded serine/threonine kinase, protein kinase 1 (PK1), was essential for hyperphosphorylation. Abundant post-translational modification sites on P6.9 were identified by MS, among which 7 Ser/Thr and 5 Arg phosphorylated sites were PK1-dependent. Mutation of these Ser/Thr sites dramatically reduced the transcription of the very late viral genes and viral infectivity, indicating that the PK1-mediated phosphorylation of P6.9 contributes to viral proliferation. Our data suggest that a potential "code" might exist in the sophisticated post-translational modification of the viral protamine-like protein and that this code is involved in viral gene transcription and infection.

Contributed Papers Tuesday 10:30 – 11:30

Bacteria 2

CONTRIBUTED PAPER Tuesday 10:30 **61**

Screening *Bacillus thuringiensis* strains with unapparent crystals

*Changlong Shu*¹, *Xuwen Zhang*^{1,2}, *Neil Crickmore*³, *Fuping Song*¹, *Jie Zhang*¹

¹State Key Laboratory of Biology for Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, P. R. China; ²School of Life Science, Northeast Agricultural University, Harbin, P. R. China; ³School of Life Sciences, University of Sussex, Falmer, Brighton, UK

Correspondence: jzhang@ippcaas.cn

The *Bacillus thuringiensis* (Bt) is an entomology pathogen, during the sporulation process, the Cry proteins accumulated in Bt mother cells can form parasporal crystalline inclusions and contribute to Bt insecticidal activities. Currently, during strain isolation process, the recognizant of Bt still based on microscopic morphology observe of crystals. As several Cry proteins been reported lacks of crystal forming domain as well as some of cry gene were silencing, the current method may missing these crystal unapparent Bt isolates. Therefore, new methods to recognize these crystal unapparent Bt strain is needed. Here we illustrated a method using cry gene conserved primers PCR test

instead of microscopic morphology observation to recognize Bt strains. By this method, 79 Bt isolates including 43 with no or unapparent crystals were screened out. The 43 isolates with no or unapparent crystals will miss in the microscopic morphology observation method. 9 of the 43 isolate shown toxicity to Lepidoptera insects. One of the Lepidoptera insects' toxic isolate was sequenced by 454 Genome Sequencer FLX™ System, and a cry1Ac mutation gene was found. This cry1Ac mutation gene can express but cannot form apparent crystals in Bt. This mutation not affects its toxicity, however, it increase the Bt spore formation rate, with may benefit's Bt environmental adaptability. And, that may be the reason why there was such a high ratio unapparent crystal Bt isolate.

CONTRIBUTED PAPER Tuesday 10:45 **62-STU**

In search for novel bioactive molecules: Genome mining of the entomopathogenic bacterium *Photorhabdus luminescens sonorensis* (Gamma-Proteobacteria: Enterobacteriaceae)

Rousel A. Orozco, S. Patricia Stock.

Department of Entomology, University of Arizona.
Tucson, AZ, USA

Correspondence: rouselo@email.arizona.edu

Microbial genome mining has become a worldwide approach for the discovery of novel compounds with biological activity. In this respect, insect pathogenic bacteria in the genus *Photorhabdus* are viewed as a rich source of diverse classes of small molecules with antimicrobial activity among other properties. One of our research foci is on *Photorhabdus luminescens sonorensis* (PLS), the bacterial symbiont of the nematode *Heterorhabditis sonorensis*. In particular, we are interested in investigating secondary metabolites which are natural products with great promise for application in agriculture. We have recently completed the sequencing of PLS and are currently annotating its genome and conducting an in depth bioinformatic analysis to identify and characterize biosynthetic gene clusters. Initial analyses have revealed the presence of more than 20 biosynthetic clusters. Some of these clusters are a apparently exclusive to this bacterium and have not been reported in other *Photorhabdus* species such as *P. luminescens* (TT01 strain), *P. temperata*, and *P. asymbiotica*. In this presentation, we will discuss functional predictions, substrate specificities, and structure of biosynthetic enzymes encoded in PLS gene clusters. Levels of sinteny conservation and phylogenetic relatedness of enzymes encoded will be also be discussed.

CONTRIBUTED PAPER Tuesday 11:00 **63**

Structural mutants of the anti-feeding prophage

Mark Hurst¹, Daria Rybakova², Alok Mitra³

¹Innovative Farm systems, AgResearch, Lincoln Research Centre, New Zealand; ²Institute of Environmental Biotechnology, Graz University of Technology, Austria; ³School of Biological Sciences, University of Auckland, Auckland, New Zealand

Correspondence: mark.hurst@agresearch.co.nz

Some strains of *Serratia entomophila* and *S. proteamaculans* (Enterobacteriaceae) cause amber disease of the New Zealand grass grub *Costelytra zealandica* (Coleoptera: Scarabaeidae). Larvae cease feeding within 2-3 days of ingestion of pathogenic strains of either *S. entomophila* or *S. proteamaculans*. The cessation of feeding virulence determinant termed Afp for anti-feeding prophage is encoded on a large 153,404-bp conjugative plasmid termed pADAP. The Afp is encoded by a cluster of 18 genes, with products of these genes combining to form a virus like particle that is a bullet-shaped toxin-delivery apparatus similar in

appearance to the R-pycocins of *Pseudomonas aeruginosa*. Morphologically it resembles the sheathed tail of bacteriophages such as T4, including a baseplate at one end. It also shares features with the type VI secretion systems. A 3 day LD₅₀ of approximately 500 cell free Afp particles per larva is required to cause cessation of feeding in *C. zealandica*. Genes (*afp1-18*) encoding components of Afp were expressed and their products purified allowing morphological assessment of the products by transmission electron microscopy (TEM). Several constructs and mutants have been shown to produce aberrant sheathless structures and Afp-like assemblies that are of shorter lengths, allowing us define the function of Afp16 and Afp14 as a sheath maturation protein and a length determining protein, respectively. This information, combined with bioinformatics data and TEM observations has allowed us to propose a model on the maturation of the Afp assembly.

CONTRIBUTED PAPER Tuesday 11:15 **64**

Loops replacements in domain II of Cry1Ab toxin with gut binding peptides alter insecticidal activity against the rice brown planthopper, *Nilaparvata lugens* (Stål)

Shao Ensi¹, Liu Sijun², Lin Li¹, Guan Xiong¹

¹Key Laboratory of Biopesticide and Chemical Biology, Ministry of Education, Fujian Agriculture and Forestry University, 350002 Fuzhou, Fujian, PR China; ²Department of Entomology, Iowa State University, Ames, IA 50011, USA

Correspondence: guanxfafu@126.com

Cry toxins derive from *Bacillus thuringiensis* (Bt) have been widely used in the management of lepidopteran, coleopteran, dipteran pests and nematode. Along with the widely planting of Bt transgenic crops, target pests will be effectively controlled. However, insecticidal spectrum of Cry toxins is limited. Seldom reports concerned to the toxicity from Cry toxins against hemipteran pests such as aphids, planhoppers and leafhoppers. As a result, after the expansion of Bt transgenic crops, hemipteran pests will only be managed by the use of traditional chemical pesticides and the deterioration of these Bt nontarget pests will increase. In our research, we screened a *N. lugens* gut binding peptide P2S and brush border membrane vesicles (BBMV) binding peptide P1Z. Then three receptor-binding loops in Cry1Ab domain II were replaced by P2S or P1Z respectively. After screening, one novel Cry toxin Cry1Ab-2S with loop 2 be replaced by P2S was selected. Cry1Ab-2S was the most stable novel toxin after the treatment by *N. lugens* gut proteases and had the strongest binding activity with *N. lugens* BBMV. Bioassay results showed that toxicity of Cry1Ab-2S against *N. lugens* nymphs were ~13 folds higher than Cry1Ab toxin (LC₅₀=17.47µg/mL). Observation of *N. lugens* gut ultrastructure showed that gut microvilli of *N. lugens* fed by Cry1Ab-2S was obviously damaged. This research provides a new method for molecular modification of Cry toxins target on specific pests. Furthermore, result of this research will help the understanding of mechanism of Cry toxins in hemipteran insects.

CONTRIBUTED PAPERS Tuesday 10:30 – 11:30

Diseases of Beneficial Invertebrates 2

CONTRIBUTED PAPER Tuesday 10:30 **65-STU**

White spot syndrome virus (WSSV) infection: Understanding pathways of infection and opportunities for treatment from the perspective of a resistant species

Bas Verbruggen, Lisa K. Bickley, Eduarda M. Santos, Charles R. Tyler, Grant D. Stentiford, Kelly S. Bateman, Ronny van Aerle
Exeter University, College of Life and Environmental Sciences,
United Kingdom

Correspondence: bv213@exeter.ac.uk

WSSV is the viral pathogen that causes White Spot Disease (WSD) in a wide range of crustacean hosts. In Penaeid shrimp, mortality rates cause \$ billions in annual losses to the shrimp aquaculture industry. Currently there is no treatment for WSD. Previous studies have shown that the shore crab (*Carcinus maenas*) is relatively resistant to WSD. In a novel approach, we are investigating molecular mechanisms of resistance in this species to inform on new opportunities for treatment in susceptible species. Our approach requires that we generate genomic resources for this work. We have therefore sequenced RNA samples from 12 individual *C. maenas* tissue types on an Illumina HiSeq platform and assembled a transcriptome encompassing approximately 212,000 transcripts; representing 62,000 unique annotated genes. The transcriptome was mined to identify genes from innate immune system pathways including melanization, JAK-STAT and Toll signalling and documented their expression profiles across individual tissues. Additionally we assembled a draft genome scaffold for *C. maenas*, approximately 1 Gb in size. This genome assembly is used to characterize regulatory elements for expressed transcripts, repeat structures and miRNAs. Currently we are investigating the temporal transcriptomic and miRNA dynamics of *C. maenas* exposed to WSSV over a period of 28 days post-infection. Preliminary analysis has identified a number of differentially regulated miRNAs that target Rab7, an important player in WSSV infection. Together, these data will allow us to elucidate how WSSV and host pathways interact, and their relationship to resistance. This information opens up new opportunities to the development of WSD treatments.

CONTRIBUTED PAPER Tuesday 10:45 **66**

Using genomics to identify host-pathogen interactions following white spot syndrome virus infection

Lisa K. Bickley¹, Bas Verbruggen¹, Kelly S. Bateman², Grant D. Stentiford², Charles R. Tyler¹, Eduarda M. Santos¹, Ronny van Aerle²

¹College of Life and Environmental Sciences, Exeter University, UK;

²Centre for Environment, Fisheries and Aquaculture Science (CEFAS), Weymouth, UK

Correspondence: L.K.Bickley@exeter.ac.uk

White Spot Syndrome Virus (WSSV) has emerged as one of the most prevalent and widespread viruses in the marine environment and is highly virulent in penaeid shrimp, a globally important aquaculture species. Recent studies demonstrated that European decapods show widely differential susceptibility to WSSV. Experimental infections of the shore crab (*Carcinus maenas*) with WSSV have found limited disease or mortality. This suggests *C. maenas* may be naturally resistant to WSSV, potentially offering an opportunity to study how this resistance is mediated.

In this project, we infected *C. maenas* with WSSV to investigate temporal changes in both the transcriptomic and miRNA responses up to 28 days post-infection.

We generated the necessary genomic resources, including a reference transcriptome and a draft genome scaffold (discussed in the abstract submitted by Verbruggen *et al*). Bioinformatics analyses identified relevant immune-response pathways and suggest the endocytic pathway as an important regulator of the response to WSSV in this species. In particular, a number of differentially expressed miRNAs were identified that may target

Rab7; a regulator of intracellular vesicle trafficking that has previously been shown to bind to WSSV envelope protein. Differential expression of Rab7 following virus infection has been confirmed via QPCR, further supporting its role in the response to WSSV in this species. Further analysis of generated RNA-Seq data is revealing other pathways that may be important in the response of *C. maenas* to WSSV infection. We will discuss how this is bringing further understanding to host-pathogen interactions and how we envisage this to inform on new opportunities for treatment in susceptible species.

CONTRIBUTED PAPER Tuesday 11:00 **67-STU**

Effect of temperature on the immune, clinical and tissue response of American lobster (*Homarus americanus*) experimentally infected with white spot syndrome virus

Louise-Marie D. Roux^{1,2,3}; Philip J. Byrne^{2,4}, K. Fraser Clark^{1,3}, Spencer J. Greenwood^{1,3}, Glenda M. Wright¹, Dorota W. Wadowska⁵

¹Department of Biomedical Sciences, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown PE, Canada;

²Gulf Biocontainment Unit, Department of Fisheries and Oceans Canada, Charlottetown, PE, Canada; ³AVC Lobster Science Centre, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown PE, Canada;

⁴Department of Pathology and Microbiology, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown PE, Canada; ⁵Electron Microscopy, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown PE, Canada

Correspondence: Lroux@upepei.ca

Water temperature influences basic life history traits of the American lobster (*Homarus americanus*) such as growth, reproduction and migration. Yet, relatively little is known about the effects of water temperature on the immune response of *Homarus americanus*. At 20°C, the American lobster produces a targeted immune response to intramuscular injection of White Spot Syndrome Virus (WSSV). However, under natural pathways (consumption trials at 10°C) no infection or disease occurs. This study will investigate the constraints imposed by a range of temperatures on the immune response of American lobster to experimental WSSV injection. A combination of light and electron microscopy will be used to examine tissue response to WSSV, as well as for evidence of viral replication. In addition, health assessments including haemocyte concentration and qPCR of haemolymph will be used to monitor the host response over time and to determine presence of viral infection, respectively. Gene expression analysis will use a lobster specific microarray and RT-qPCR to identify differentially expressed immune and temperature related genes in the host. Initial results from this study involving changes in haemocyte concentration (as an indicator of health) corroborate previous work in which experimental establishment of infection and disease only occurs at higher temperatures. The use of a range of experimental temperatures may help to explain why post-injection infection and disease only occur at higher temperature. Results from the study will broaden our understanding of how temperature influences immune response and provide insight into host pathogen interactions in the American lobster.

CONTRIBUTED PAPER Tuesday 11:15 **68**

Paramyxia: Emergence of an enigmatic class of invertebrate parasites

David Bass^{1,2}, Georgia Ward^{1,2}, Rose Kerr¹, Martyn Bennett¹, Rosaline Hulse, Grant D. Stentiford¹

¹Centre for Environment, Fisheries, and Aquaculture Science (Cefas), Barrack Road, The Nothe, Weymouth, Dorset DT4 8UB, UK; ²Department of Life Sciences, The Natural History Museum, London SW7 5BD

Correspondence: david.bass@cefas.co.uk

Paramyxians cause economically significant mortalities of bivalves, including Marteiliellosis in the European oyster *Ostrea edulis* and QX disease in the Sydney rock oyster *Saccostrea glomerata*, cause disease in crabs, and have been implicated in modification of sexual status in amphipods. The class as a whole is very poorly known: they are very genetically divergent and little is known about their host ranges or biology outside of the hosts of economic concern. However, novel paramyxian lineages are increasingly being detected in a wide range of invertebrate hosts, and their star is clearly in the ascendancy. In February 2015 the first Paramyxian Working Group Meeting was held in Spain, attracting delegates from around the world. We present a fully comprehensive paramyxian phylogeny, clarifying the taxonomy of the group, and report results from environmental DNA (eDNA) and other molecular studies to further investigate paramyxian diversity, ecology, and host affiliations.

WEDNESDAY – August 12th

SYMPOSIUM Wednesday 8:00 – 10:00

Advances in Host and Insect Virus Genomics

SYMPOSIUM PAPER Wednesday 8:00 **69**

Macro- and micro-evolutionary trends in baculoviruses

Johannes A. Jehle, Laurin R. Monnheimer, Gianpiero Gueli Alletti, Jörg T. Wennmann

Institute for Biological Control, Federal Research Centre for Cultivated Plants, Julius Kühn Institute, Darmstadt, Germany

Correspondence: johannes.jehle@iki.bund.de

Baculoviruses are most probably the largest group of insect-pathogenic dsDNA viruses worldwide. They co-evolved with their insect hosts in the orders Lepidoptera, Diptera, and Hymenoptera. They are classified in four genera, the *Alpha*-, *Beta*-, *Gamma*-, and *Deltabaculovirus*. Phylogenetically, baculoviruses are related to nudiviruses, bracoviruses, hytrosaviruses and most probably whispoviruses. All these viruses share a number of highly conserved structural proteins involved in the infection process, transcription, and DNA replication. Nearly 60 baculovirus genomes have been completely sequenced, so far. All sequenced baculovirus genomes share 37 conserved gene homologues, called “core genes”. It is assumed that these core genes are ancient and represent the footprint of a common ancestor of all recent baculoviruses. In an evolutionary process, the genomes of baculoviruses have been further shaped by gene insertion through lateral gene transfer from other viruses, microorganisms and hosts, genome rearrangements such as inversions, translocations of single genes or gene blocks, as well as deletions and duplications of single genes. On nucleotide level, heterogeneity is caused by nucleotide transitions, transversions, and indel mutations. Based on the arrangement of 37 baculovirus core genes in fully sequenced baculoviruses, we try to retrace major trends in genome evolution of baculoviruses and related dsDNA viruses, providing some hints about the possible genome

arrangement of the common baculovirus ancestor. Using the examples of the *Agrotis*-specific nucleopolyhedroviruses as well as single isolates of *Cydia pomonella* granulovirus, micro-evolutionary trends can be delineated and correlated with alignment based phylogenies on species and isolate level of baculoviruses.

SYMPOSIUM PAPER Wednesday 8:24 **70**

Alphabaculoviruses: Host transcriptome responses to infection

Yun-Ru Chen^{1,2}, Silin Zhong², Zhangjun Fei¹, Zhaofei Li³, Ping Wang⁴, Gary W. Blissard¹

¹Boyce Thompson Institute at Cornell University, Ithaca, New York, USA; ²State Key Laboratory of Agrobiotechnology, The Chinese University of Hong Kong, Hong Kong, China; ³Key Laboratory for Applied Entomology, Northwest A&F University, Yangling, China; ⁴Department of Entomology, Cornell University, New York State Agricultural Experiment Station, Geneva, New York, USA

Correspondence: gwb1@cornell.edu

The baculovirus genome has a large coding capacity, and mediates a precisely ordered and regulated series of events in a complex infection cycle in the host cell. Through viral gene expression, and the resulting host cell responses, the virus controls the infection cycle at both the cellular and organismal levels. Viral infection dramatically modifies subcellular and cellular architecture, function, and metabolism. To begin to understand how these complex virus-insect interactions are controlled, we performed RNA-seq based studies in cultured *Trichoplusia ni* cells infected with the *Autographa californica* Multiple Nucleopolyhedrovirus (AcMNPV), quantifying transcripts from viral and cellular genes through the infection cycle. Viral transcripts, as a group, increase steadily and become the predominant cellular mRNAs between 12 and 18 hours post infection (h pi). In concert, the vast majority of host transcripts are down regulated through the infection cycle. However, we identified a substantial number of host genes that are up-regulated temporarily between 0 and 6 h pi, and a very small number of host genes that are induced or substantially up-regulated through 24, 36, or 48 h pi. We also performed a parallel study with an AcMNPV virus containing a knockout in the important *late expression factor 5 (lef-5)* gene, a gene that is critical for late gene transcription, but not for viral DNA replication. By comparisons with WT AcMNPV, we are able to identify viral and host transcriptome effects that result from expression of viral early-phase genes, and thus to differentiate between effects of early and late viral genes.

SYMPOSIUM PAPER Wednesday 8:48 **71**

Polydnaviruses: From discovery to current insights

Michael R. Strand

Department of Entomology, University of Georgia, Athens, GA, USA

Correspondence: mrstrand@uga.edu

The Polydnaviridae was recognized by the International Committee on Taxonomy of Viruses (ICTV) in 1991 as a family of viruses with large double-stranded DNA genomes that are associated with parasitoid wasps. Historically, polydnaviruses have received limited attention in the literature because their life cycle and unusual biology makes them difficult to work with. Yet recent advances have elevated interest in polydnaviruses precisely because their unusual biology sheds interesting light on virus evolution and what the essential qualities of viruses are. As part of this Virus Division symposium on host and insect virus genomics, I will first summarize the early literature that led ICTV

to recognize polydnviruses. I then will discuss the more recent literature, founded on genomics and experimental data, and what these results reveal about polydnvirus evolution and function.

SYMPOSIUM PAPER Wednesday 9:12 **72**

Dicistrovirus - hijacking the host translational machinery

Eric Jan

Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, BC Canada

Correspondence: ej@mail.ubc.ca

Dicistroviruses are monopartite, plus strand RNA viruses that primarily infect arthropods of agricultural and economic significance including honey bees, fire ants and shrimp. Members of this family include Cricket paralysis virus, Taura syndrome virus and Israeli acute paralysis virus. Dicistrovirus infection results in a shutoff of host protein synthesis concomitant with preferential translation of viral proteins. The dicistrovirus utilizes a cis-acting RNA element called an internal ribosome entry site (IRES), which adopts a unique structure that directly recruits the host ribosome without the need of any translation factors and initiates translation from a non-AUG codon. Structural and biochemical analyses have revealed that the IRES partially mimics a tRNA to occupy the conserved ribosomal core in order to manipulate the ribosome for viral protein synthesis. Recent work demonstrate that a subset of dicistroviruses (honey bee and fire ant viruses) contain an IRES that can direct translation in two different reading frames thus leading to expression of a hidden +1 overlapping open reading frame. Our work suggests that distinct structural conformations of the IRES affect translational reading frame selection. These studies have revealed a novel viral strategy that uses an unprecedented RNA element that mimics a natural tRNA to directly hijack the host ribosome and initiate translation in alternative reading frames in order to increase the coding capacity of the viral genome.

SYMPOSIUM PAPER Wednesday 9:36 **73**

Insect metagenomics-based discovery of novel, small RNA viral genomes

Sijun Liu, Yuting Chen, Bryony C. Bonning

Department of Entomology, Iowa State University, Ames, IA 50011

Correspondence: slu@iastate.edu

In recent years, sequences derived from more than a hundred novel RNA viruses have been found from insects and insect cell lines in the absence of disease symptoms. The vast majority of these virus genome sequences were identified on assembly and analysis of Next Generation Sequencing (NGS) data. Caution is required for interpretation of such sequence discoveries on the bases that 1) the presence of virus-derived sequences does not necessarily mean that a virus is present, and 2) viruses associated with a given insect do not necessarily infect that insect. It is therefore inappropriate to ascribe virus sequences discovered through the metagenomics approach to viruses of a particular host without experimental confirmation. Evidence for replication in the putative host is required before the virus can be assigned to that host. By analyzing Illumina transcriptome sequence data, we identified several novel small RNA viruses from the soybean aphid (*Aphis glycines*), Western corn rootworm (WCR) (*Diabrotica virgifera virgifera*) and various stink bug species. We used multiple approaches to verify these viruses, including RT-PCR and Sanger sequencing to confirm assembled viral sequences, 5'- and 3'-RACE for acquisition of the complete viral genome sequence, isolation of virions for visualization by TEM, characterization of

viral proteins, examination of tissue tropism, assessment of virus transmission routes, susceptibility of cell lines to infection, and small RNA sequencing to assess the role of RNA interference in degradation of viral RNA. A combination of NGS technology, bioinformatics analysis and conventional virological methods are required for the discovery and confirmation of insect viruses.

CONTRIBUTED PAPERS Wednesday 8:00 – 9:45

Microbial Control 2

CONTRIBUTED PAPER Wednesday 8:00 **74**

Potential of Aprehend™ (*Beauveria bassiana*) as a bed bug control agent

Alexis M. Barbarin¹, Nina E. Jenkins², Giovanni Bellicanta², Matthew B. Thomas^{2,3}, Coby Schal¹

¹Department of Entomology, North Carolina State University, 100 Derieux Pl. Campus Box 7613, Raleigh, NC, 27695, USA;

²Department of Entomology, Penn State University, Merkle Lab University Park, PA 16802, USA; ³Center for Infectious Disease Dynamics, Penn State University, Merkle Lab, University Park, PA 16802, USA

Correspondence: alexis_barbarin@ncsu.edu

Pyrethroids have been a mainstay chemistry in bed bug control, yet resistance to pyrethroid insecticides is prevalent and high in field populations of bed bugs. A series of bioassays was conducted on the human bed bug *Cimex lectularius*, to evaluate the efficacy of Aprehend™, a *Beauveria bassiana* based residual insecticide, against a pyrethroid-susceptible and three field-collected strains of bed bugs. Aprehend™ was applied to box spring batting at a rate of 3×10^6 conidia/cm² (draft label rate) using a modified sprayer. Suspend® SC (AI deltamethrin) was applied to box spring batting at both the low (maintenance, 0.03% active ingredient) and high (cleanout, 0.06% active ingredient) labeled rates, using a Potter Tower. All four strains were exposed to sprayed substrates for 15 minutes, then transferred to an unsprayed environment and monitored for mortality over a period of 14 days. Assays revealed that the three field-collected strains were resistant to Suspend® SC, whereas the laboratory strain was susceptible to Suspend® SC. A 15 min exposure to Aprehend™ caused rapid infection and mortality in bed bugs from all four strains. These results demonstrate that relatively short exposure to Aprehend™ results in mortality of pyrethroid-resistant bed bugs, highlighting the potential utility of *Beauveria bassiana* as an effective tool within an integrated bed bug management toolkit.

CONTRIBUTED PAPER Wednesday 8:15 **75**

***Beauveria bassiana* for the control of stored grain pests**

Aoife B. Dillon¹, Clare G. Storm¹, Freya C. L. Scoates¹, Adam J. Nunn¹, Sue E. Harris¹, Maureen E. Wakefield², Belinda Luke³, Bryony Taylor³, Dave Moore³, Pierre M Grammare⁴, Olivier Potin⁴

¹Exosect Ltd, Leylands Business Park, Colden Common, Winchester, Hampshire, SO21 1TH, UK; ²The Food and Environment Research Agency, Sand Hutton, York, YO41 1LZ, UK;

³CABI Europe-UK, Bakeham Lane, Egham, Surrey, TW20 9TY, UK;

⁴Agrauxine, 18 route de Mauvières, Zi Tivoli, Loches, France

Correspondence: Aoife.Dillon@exosect.com

Concerns over resistance development, residues and environmental impacts together with changes in EU legislation have led to a decline in available pesticides to protect stored food. Alternative products for pest control are required to maintain levels of food production and anticipate future demand. Exosect

have been working with research partners (The Food and Environment Research Agency and CABI) and expert manufacturers (Agrauxine) over the past nine years to develop the first biological product for the EU grain market based on the entomopathogenic fungi *Beauveria bassiana* (isolate IMI389521). This development has been supported by a number of grant-aided projects from the UK government including the recent Agri-Tech award to develop a formulation for grain admixture. Exosect submitted a dossier to the Dutch authorities in 2014, and are sharing costs with Agrauxine, to register the isolate for use. This will be followed up with product submissions for both admixture and pre-harvest building treatments in 2016. The pre-harvest treatment achieved high levels of efficacy (>90% at 14 d) against beetle pests *Oryzaephilus surinamensis* and *Cryptolestes ferrugineus* in a series of regulatory standard on-farm trials. The admixture formulation in development is significantly efficacious against adults of the aforementioned beetle species as well as *Rhyzopertha dominica*, *Sitophilus* spp. and the immature life stages of hard-to-control *Tribolium confusum*. Exosect have an exclusive license to commercialise the isolate for stored commodity protection and seek partners, both within the EU and US, for the development and commercialisation of structural and admixture treatments.

CONTRIBUTED PAPER Wednesday 8:30 **76-STU**

Semi-field trials and tribulations

Brian Lovett¹, Etienne Bilgo², Abdoulaye Diabate², Raymond St. Leger¹

¹University of Maryland, College Park; ²Centre Muraz, Burkina Faso

Correspondence: lovettbr@umd.edu

Transitioning any lab experiment into the field is a critical and often onerous undertaking for a research program. Transgenic fungi engineered to target mosquitoes deserve additional forethought. In collaboration with the Centre Muraz in Burkina Faso, we have built a malaria-sphere and are conducting semi-field trials with *Metarhizium anisopliae* armed with anti-plasmodium toxins. My work aims to set a precedent for such translational research. These trials offer the unique opportunity to compare the efficacy of transgenic and wild-type fungi at modulating transmission of the malaria parasite in field conditions. In addition, we are surveying the local fungal community for native *Metarhizium* candidates to use in biocontrol. This research is a coordinated international effort to establish transgenic entomopathogenic fungi as a practical method for vector control; this merits discussion as a case study illustrating the scientific, regulatory and social hurdles researchers should anticipate as they pursue real world applications of new technologies. With several long-term goals on the horizon, our results thus far include the completion of an experimental facility, the establishment of an entomopathogen research program in Burkina Faso, accuracy evaluation of semi-field conditions, and comparisons of field versus lab applications of fungi.

CONTRIBUTED PAPER Wednesday 8:45 **77**

A clean start strategy for seasonal poinsettia cuttings

Michael Brownbridge¹, Rose Buitenhuis¹, Graeme Murphy², Taro Saito, Angela Brommit¹

¹Vineland Research and Innovation Centre, 4890 Victoria Avenue North, Box 4000, Vineland Station, Ontario L0R 2E0, Canada;

²bioLogical Control Solutions, Welland, Ontario L3C 3G9, Canada
Correspondence: michael.brownbridge@vinelandresearch.com

In 2012, poinsettia cuttings shipped into Ontario from offshore production facilities carried large numbers of Bemisia whitefly eggs and nymphs. Although releases of parasitoids (*Encarsia formosa*, *Eretmocerus mundus*) proceeded as normal, they failed to regulate whitefly populations due to pesticide residues and higher-than-usual starting populations of whiteflies. Endemic whitefly resistance meant that growers had limited (insecticide) options available to them to mitigate the pest; new early season tools are required that can be applied to cuttings to prevent pest populations developing beyond the 'capacity' of the parasitoids used, and to ensure that effective biological control systems can be maintained through the production cycle. Previous research showed that immersion of infested cuttings in a combination of BotaniGard® (*Beauveria bassiana*) and insecticidal soap prior to sticking can efficiently suppress early season infestations, mitigating larger pest problems later in the season. To ensure compatibility with subsequent releases of biocontrol agents, and improved overall efficacy, trials were undertaken to assess different strategies where the 'Clean Start' approach was taken, and to validate the utility of this technique on a commercial scale. Results from these trials will be presented.

CONTRIBUTED PAPER Wednesday 9:00 **78**

Selection and characterization of Colombian entomopathogenic fungi against *Cerotoma tingomariana*

Erika Grijalba, Carlos Espinel, Adriana Santos, Cindy Mejia, Carolina Ruiz

Biological Control Laboratory, Colombian Corporation for Agriculture Research, CORPOICA. Mosquera, Colombia
Correspondence: egrijalba@corpoica.org.co

Cerotoma tingomariana (Coleoptera: Chrysomelidae) is the most limiting pest among the Chrysomelids of soybean in Colombia, due to its high frequency and distribution. This insect can affect seeds, nodules, leaves and roots, reducing the yield crop and is controlled with insecticides that some of them are forbid in USA or Europe. The aim of this work was to select and characterize an efficient entomopathogenic fungus on *C. tingomariana*. Seven isolates of *Beauveria bassiana* (Bv) and six isolates of *Metarhizium anisopliae* (Mt) were biological testing on laboratory. Mt isolates showed efficiency under 50%. Isolates Bv060 and Bv003 showed an efficiency of 100%. These isolates were tested on different temperatures (5°C, 15°C, 25°C, 30°C, 35°C), pH values (3, 5, 7, 9) and tolerance to UVB radiation (302 nm) by measuring germination, radial growth and Colony Formate Unit (CFU). In the UVB radiation test, Bv060 reduced the conidia viability between 75% and 80%, and Bv003 between 65% and 66%. At 5 and 9 pH value, the two isolates showed germination higher than 90% and the faster rate of radial growth. Bv003 showed the best growth at 15°C and 25°C and Bv060 at 25°C and 30°C. Lethal concentration 90 of Bv060 and Bv003 were determined in 5,8x10⁷ conidia/ml and 1,3x10⁵ conidia/ml, respectively. A compatibility test with excipients was performed by measuring germination (%) and (CFU). The isolated Bv060 was compatible with six excipients and Bv003 with seven excipients, which will be used to develop a granulated and an emulsifiable concentrate.

CONTRIBUTED PAPER Wednesday 9:15 **79**

Obstacles and opportunities for the use of biopesticides in conventional agriculture

Surendra K. Dara

University of California Cooperative Extension, San Luis Obispo, CA, USA

Correspondence: skdara@ucdavis.edu

Biopesticide industry has been showing a consistent growth for the past few years and is projected to reach \$6 billion by 2020. In spite of many major chemical companies investing in biopesticide production, their use in conventional agriculture is very limited in many cropping systems in California. Biopesticides can play an important role in integrated pest management. Understanding their modes of action, influence of various agronomic practices, impact of environmental conditions, and interaction with various pest management options will help growers and researchers to explore their full potential. Various challenges and opportunities in using biopesticides in conventional agriculture will be discussed.

CONTRIBUTED PAPER Wednesday 9:30 **80-STU**

Evaluation of the entomopathogenic fungi *Isaria* sp. as a biocontrol agent against pest insects in greenhouses

*Katharina Saar*¹, *Jasmin Philipp*², *Edgar Schliephake*², *Andreas Leclerque*³, *Manuel Werner*¹, *Johannes A. Jehle*², *Dietrich Stephan*¹

¹Institute for Biological Control, Julius Kühn Institute, Darmstadt, Germany; ²Institute for Resistance Research and Stress Tolerance; Julius Kühn Institute, Quedlinburg, Germany; ³Institute for Microbiology and Biochemistry, Geisenheim University, Geisenheim, Germany

Correspondence: katharina.saar@jki.bund.de

The use of the entomopathogenic fungus *Isaria* sp. in an integrated pest management strategy offers a promising approach for pest insect control in greenhouses. One necessary prerequisite is to find highly virulent and effective strains. Virulence of ten strains of *Isaria* sp., obtained from different geographical origins and hosts were compared and discriminated by their metabolic profiles and virulence against various pest insects which are up to now mainly controlled by synthetic insecticides. Using different methods, the efficacy and LC₅₀ of different *Isaria* strains against *Spodoptora exigua* (Lepidoptera) and *Bemisia tabaci* (Hemiptera) were studied. The mortality rate differed depending on the isolate from 30 % to 76 %. Additionally to this mortality effects, the feeding (sucking) behaviour of *Isaria*-infected, by application of 1x10⁶ conidia/ml, and non-infected adult whiteflies *B. tabaci* was studied, using the electrical penetration graph method (EPG). The feeding of whiteflies not only causes direct plant damages but it is also important for plant virus transmission. First results of the comparisons concerning differences in the penetration frequencies and changes within the waveform, monitored by EPG, durations will be shown. Currently, different molecular methods, such as RFLP and others, are used for the characterization of virulence factors of *Isaria* sp. and its influence on different activity patterns of *B. tabaci* as mentioned above.

CONTRIBUTED PAPERS Wednesday 8:00 – 9:45

Nematodes 1

CONTRIBUTED PAPER Wednesday 8:00 **81**

Selection of lipid and protein sources for liquid fermentation of entomopathogenic nematodes

*Luis G. Leite*¹, *David I. Shapiro-Ilan*², *Selcuk Hazir*³, *Mark A. Jackson*⁴

¹Instituto Biológico, APTA, CP 70, Campinas, SP 13001-970, Brazil; ²USDA-ARS, SEFTNRL, Byron, GA 31008, USA; ³Adnan Menderes University, Faculty of Arts and Sciences, Department of Biology, Aydin-Turkey; ⁴USDA-ARS, NCAUR, Peoria, IL, 61604, USA

Correspondence: lgleite@biologico.sp.gov.br

Despite great progress in the past couple of decades, entomopathogenic nematodes production in liquid fermentation still requires improvements to maximize efficiency, yield and nematode quality. Thus, this study was aimed at developing more suitable liquid media for mass production of *S. feltiae*. In this phase of the project, we focused on selection of lipid and protein sources, which are critical requirements for nematode growth. Two experiments were conducted. In the first experiment, seven lipid sources (corn oil, palm oil, fish oil, peanut oil, sun oil, canola oil and pork lard) were tested at a concentration of 4%; media contents included also yeast extract (2.3%), NaCl (0.5%) and egg yolk (1.23%). In the second experiment, six protein sources (egg yolk, yeast extract, egg white, soy extract, beef extract and fish collagen) were tested at a concentration of 1.25%; media contents included also yeast extract (2.3%) and NaCl (0.5%). Also, for the second experiment, one group of treatments was run with corn oil and one group with peanut oil (both at 4%). For each treatment, 3 replications were included. Each replication, consisting of a 250 ml Erlenmeyer flask containing 50 ml of liquid media, was kept in a shaker for 28 days. Both experiments were repeated in time. For the lipid experiment, pork lard provided the lowest nematode yield, while peanut oil provided the highest. For the protein experiment, the yeast extract provided the lowest nematode yield, whereas egg yolk and egg white provided the highest.

CONTRIBUTED PAPER Wednesday 8:15 **82**

An unsolvable root maze: Elevated atmospheric CO₂ increases root architectural complexity and reduces entomopathogenic nematode infectiousness

A. Srikumar, *L. Demarta*, *S.N. Johnson*, *L. Hiltbold*

Hawkesbury Institute for the Environment, University of Western Sydney, Penrith, NSW, Australia

Atmospheric CO₂ concentration is now higher than it has ever been over the last 400,000 years. Because CO₂ concentration is typically 10-fold higher in soil, direct effect of elevated CO₂ (eCO₂) on rhizospheric trophic cascades is supposed to be limited. However, eCO₂ impacts root architectural complexity. Little is known about the physical role of root on entomopathogenic nematode (EPN) foraging behavior; yet, increasing root architecture complexity weakens the infectiousness of the EPN *Heterorhabditis megidis*. To evaluate the impact of atmospheric CO₂ concentration on EPN infectiousness, maize plants were grown in either ambient or eCO₂ environments before insect baits were placed at the bottom of each pot. The number of adult of the EPN *Heterorhabditis bacteriophora* present in baits was recorded 36h after their injection. Atmospheric eCO₂ favored EPN infectiousness in pots with soil only. However, the dramatic increase in root architecture of plants grown at atmospheric eCO₂ significantly reduced EPNs infectiousness; less EPN adults were recovered from these baits than from baits at the bottom of plants grown at ambient atmospheric CO₂. These results add to the scarce data on the impact of root complexity on EPN infectiousness. Even though it is often thought that atmospheric eCO₂ has minimal impact on soil communities, especially in agricultural systems, the increase in atmospheric CO₂ concentration negatively affected the present belowground trophic cascade, possibly leading to the reduction of specific soil ecological services in soil-dwelling insect pest management.

CONTRIBUTED PAPER Wednesday 8:30 **83-STU**

Investigating *Deladenus siricidicola* Kamona (Tylenchida: Neotylenchidae) to control *Sirex noctilio* (Hymenoptera: Siricidae) In North America

Isis A. L. Caetano, Ann E. Hajek

Cornell University, Department of Entomology, Comstock Hall,
129 Garden Ave, Ithaca, New York, United States
Correspondence: ial26@cornell.edu

Sirex noctilio is an invasive pest in North America that kills pine trees (*Pinus* spp.). The nematode *Deladenus siricidicola* Kamona, which sterilizes *S. noctilio* females, can be mycetophagous and free living or parasitic. We tested *D. siricidicola* Kamona against *S. noctilio* to investigate how effective it can be at controlling this woodwasp in North America. In North America another strain of *D. siricidicola* that does not sterilize *S. noctilio* is present, presumably introduced with this invasive. *Deladenus siricidicola* Kamona were reared on two different strains of the fungus *Amylostereum areolatum*, one native to North America (IGS-BE) and the other (IGS-BDF) is used commercially to mass-produce the nematode in Australia. We injected Kamona into logs infested with *S. noctilio* larvae. All wasps that emerged from logs were dissected and nematode samples were taken from infected wasps. To identify the nematodes collected from wasps we sequenced the mtCO1 gene. Of the forty-seven wasps that emerged from BDF fungal-treated logs, only two wasps were infected with the Kamona strain *D. siricidicola*. Forty *S. noctilio* emerged from control logs and none of them were infected with nematodes. No wasps emerged from BE-treated logs, and these logs are currently being evaluated further by extracting larvae that might still be within the wood to detect infection with different nematode strains.

CONTRIBUTED PAPER Wednesday 8:45 **84-STU**

Interactions between parasitic nematodes and bacteria in *Drosophila*

M. A. Hanson, S. J. Perlman

Biology Department, University of Victoria, Victoria BC, Canada
Correspondence: markhans@uvic.ca

Interactions between co-infecting microorganisms can have important consequences on host susceptibility and on the evolution of parasites and pathogens. Woodland mushroom-breeding *Drosophila* are a wonderful system for studying co-infection dynamics in the wild. Important parasites of woodland *Drosophila* are *Howardula* nematodes, commonly found on decaying mushrooms. Interestingly, the Testacea group of *Drosophila* flies shows marked variation in susceptibility to *Howardula* infection. These nematodes infect fly hosts by piercing a hole in the larval cuticle with a harpoon-like stylet, after which they develop within host haemocoel. This method of entry leaves larval flies vulnerable to pathogenic bacteria that gain direct access to host haemolymph through this hole. As *Howardula* require their fly hosts to complete development for transmission, this raises intriguing questions regarding the fly's response to these unwelcome guests. To assess the effects of interactions amongst co-infecting natural enemies, we investigated the responses of Testacea group flies to nematodes and bacteria in larvae and adults. We orally infected Testacea group flies with a *Serratia* bacterium we isolated from *Drosophila*; *Serratia* is a common soil bacterium and model of intestinal infection in *Drosophila*. We later co-infected larval and adult flies with *Howardula aoronymphium* and *Serratia*, and determined their effects on host fitness. We found interesting variation in Testacea group responses to *Serratia* infection, and describe the effects of co-infection with nematodes and bacteria on host fitness.

CONTRIBUTED PAPER Wednesday 9:00 **85-STU**

Characterization of immune response genes in the parasitic nematode *Brugia malayi*

Silvia Libro, Jeremy Foster, Barton Slatko

New England Biolabs, 240 County Road, Ipswich, MA, 01938, USA
Correspondence: libro@neb.com

The filarial nematode *Brugia malayi* is one of the causative agents of lymphatic filariasis, a neglected tropical that affects millions of people worldwide. Due to the limited effectiveness of the drugs available and the absence of a vaccine, understanding the basic biology of *B. malayi* and its symbiotic association with *Wolbachia* endobacteria is a priority. At present, little is known about the mechanisms underlying *Brugia*'s immune system. This overlooked area of research is of high interest, as it can help to advance our understanding on the interaction between *Brugia* and *Wolbachia*. It has been suggested that in order to reside within the nematode's tissue, *Wolbachia* may evade the host immune system. Therefore, understanding what mechanisms are involved in *Brugia* immunity can help in identifying targets for the development of new drugs and vaccines. In order to characterize the main genetic pathways involved in *B. malayi* immunity, we performed a transcriptome analysis of adult female worms exposed to different immune elicitors, including bacterial lysates, short dsRNA, and dsDNA. Small RNA and differential gene expression analysis of untreated and immune challenged worms were used to characterize gene expression patterns associated to each type of immune insult and allowed to identify selected candidate immune genes.

CONTRIBUTED PAPER Wednesday 9:15 **86-STU**

One ring to bind them all: Is heme biosynthesis an influencing factor in *Wolbachia*-filarial nematode endosymbiosis?

Ashley N. Luck, Jeremy M. Foster, Barton E. Slatko

Genome Biology Division, New England Biolabs, 240 County Road,
Ipswich, MA 01938
Correspondence: Slatko@neb.com

Genomic sequencing has revealed that many human filarial nematodes, such *Wuchereria bancrofti*, *Brugia malayi* and *B. timori*, (causative agents of lymphatic filariasis (LF)) and *Onchocerca volvulus* (causative agent of onchocerciasis (river blindness)) contain the obligate endosymbiont, *Wolbachia*. Laboratory and human trials show that depletion of *Wolbachia* by antibiotics (e.g. doxycycline), can lead to blocking of embryogenesis, elimination of microfilariae (mf) output and adult worm killing. While the factors which are key to this obligate inter-relationship are not fully understood, genome sequencing identified a number of critical processes implicated in the host-endosymbiont interaction, one of which was heme biosynthesis. By serving as a co-factor in a number of enzymatic and biochemical processes, heme and heme regulation is essential to organism survival. Although *B. malayi* contains a functional ferrochelatase gene (the final step in the heme biosynthetic pathway and a product of lateral gene transfer), as with other nematodes they are incapable of synthesizing heme. However, the *Wolbachia* genome contains a complete and functional heme biosynthesis pathway, suggesting that *Wolbachia* may supply the host nematode with heme. Exploiting "NextGen" transcriptome and DNA sequencing, we have investigated differential expression patterns of the host and symbiont responses to heme in filarial nematodes. Numerous heme response genes (hrgs) from both organisms are differentially expressed in the presence of heme. Several show homology hrgs found in the free living nematode, *C. elegans*, that does not contain *Wolbachia*. RNAi and inhibitor

studies in *B. malayi* further suggest heme homeostasis regulation may be important in the symbiotic relationship between the two organisms and thus might be a target for filariasis control.

CONTRIBUTED PAPER Wednesday 9:30 **87-STU**

Phenotypic diversity in the virulence of the entomopathogenic bacterium *Xenorhabdus bovienii* (Gamma-Proteobacteria: Enterobacteriaceae) reveals a type VI secretion system in its pan-genome

*John G. McMullen*¹, *S. Patricia Stock*²

¹School of Animal and Comparative Biomedical Sciences, The University of Arizona, Tucson, AZ, USA; ²Department of Entomology, The University of Arizona, Tucson, AZ, USA
Correspondence: jgm2@email.arizona.edu

Entomopathogenic *Xenorhabdus* bacteria have diverse interactions with invertebrate hosts, serving both as mutualists to nematodes and as pathogens to insects. The bacteria rely on the nematode for their transmission from one insect host to another. In return, *Xenorhabdus* provide a suitable environment for nematode development and reproduction by killing the insect host and eliminating the native insect microbiota. Each *Steinernema* sp. is colonized by a specific *Xenorhabdus* sp., whereas a given *Xenorhabdus* sp. may have multiple *Steinernema* spp. hosts. The most promiscuous *Xenorhabdus* sp. known in this system is *Xenorhabdus bovienii*, which colonizes at least nine *Steinernema* spp. In this study, we used a comparative genomics approach to identify candidate virulence factors for nine *X. bovienii* strains. Virulence was determined by assessing mortality in three insect hosts: *Spodoptera littoralis*, *Spodoptera frugiperda*, and *Galleria mellonella*. Through these assays, five virulent strains and four attenuated strains were identified. Analysis of the pan-genome of this bacterium revealed a type VI secretion system (T6SS) present only in virulent strains. Preliminary results indicate the T6SS is involved in intra-bacterial competition. *In vitro* competition assays were performed to further assess T6SS role in this bacterium's virulence. Our results suggest that virulent bacteria outcompete the attenuated strains. Examining the role of this T6SS will further increase our understanding of the *Steinernema-Xenorhabdus* interaction in the insect infection process. We speculate this locus may allow *X. bovienii* to outcompete the insect microbiota, including other co-infecting *Xenorhabdus* spp. and leading to the success of *X. bovienii* in colonizing *Steinernema* nematodes.

SYMPOSIUM CROSS DIVISION Wednesday 10:30 – 12:30

Intracellular Responses to Bacteria and Bacterial Toxins

SYMPOSIUM PAPER Wednesday 10:30 **88**

Insecticidal action, cellular interactions and response of combinations of *Photorhabdus*-Insect-Related (Pir) and *Bacillus thuringiensis* Crystal (Cry) Toxins

*Anais Castagnola*¹, *Rousel Orozco*², *Kathy Teng-Nelson*³, *Don Lightner*³, *Patricia Stock*²

¹Center for Insect Science, University of Arizona, Tucson; ²Entomology Department, University of Arizona, Tucson; ³School of Animal and Comparative Biomedical Sciences, University of Arizona, Tucson
Correspondence: anais@email.arizona.edu

Recent studies have shown that PirB toxin can be coupled to Cry2A toxin and have insecticidal activity when delivered *per os* to *Spodoptera exigua*. However, the combined activity of this toxin chimera is apparently host-specific. Additionally, it has been suggested that PirB can be insecticidal without PirA as long as similar structural domains, putatively for membrane recognition, are adequately replaced. Recently, the shrimp pathogen *Vibrio parahaemolyticus*, which causes acute hepato-pancreatic necrosis disease in farmed shrimp populations, has been investigated for its toxins and its pathogenic properties. Bioassays have confirmed the pathogenic activity of PirAB-like toxins in shrimp, but until now no insect pathogenic effect has been assessed. We hypothesized that *Vibrio* PirAB-like toxins in combination with Bt Cry toxins may have effects similar to other Pir/Cry combinations. In this respect, we have further investigated interactions between Cry1Ac/2Ab toxins with Pir toxins from two *Photorhabdus* spp.: *P. luminescens*, *P. asymbiotica*, and from *V. parahaemolyticus*. Using selected insect species, we have investigated cellular components of host gut- tissue, protein and lipids, involved in the interaction with Cry and Pir toxins. We have further characterized the toxicity of PirAB with Cry1Ac/2Ab by analyzing toxin response pathways upon ingestion. Finally, we have investigated binding/receptor recognition, insecticidal motifs, structure, and homology of *Vibrio* PirAB sequence, considering a bioinformatics approach. Results of these studies will be presented and further discussed.

SYMPOSIUM PAPER Wednesday 10:50 **89**

Response to Cry1Ac intoxication in midgut cells of *Heliothis virescens* larvae

*Cris Oppert*¹, *Omaththage P. Perera*², *Thomas Lane*¹, *Margaret Staton*¹, *Heba M. Abdelgaffar*¹, *Juan Luis Jurat-Fuentes*¹

¹Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN, USA; ²USDA-ARS, Southern Insect Management Research Unit, Stoneville, MS, USA
Correspondence: jurat@utk.edu

The Cry1 insecticidal proteins from the bacterium *Bacillus thuringiensis* (Bt) target insect midgut cells. While still under discussion, the specific mechanism of enterocyte killing is associated with binding of the Cry1 proteins to receptors followed by insertion and formation of toxin-lined pores on the cell membrane. Alternative proposed models suggest the possibility of intracellular cell death pathways activated by binding of the toxin to receptors as being responsible for enterocyte death. To elucidate the importance of intracellular pathways in enterocyte death and the identification of defensive responses to the bacteria, we performed transcriptome profiling by RNAseq using midguts of *Heliothis virescens* larvae exposed to Cry1Ac or a Bt-based pesticide. Moreover, we included in the comparison *H. virescens* strains with high levels of resistance to the Cry1Ac protein due to highly reduced Cry1Ac protein binding. Gene expression comparisons between controls (untreated) or unaffected (resistant) and susceptible larvae identify intracellular pathways involved in enterocyte death as well as putative defensive mechanisms. In this presentation we will report findings from this analysis and discuss their relevance to current models of Cry1 intoxication in Lepidoptera.

SYMPOSIUM PAPER Wednesday 11:10 **90**

Syringe-like injection mechanism of bacterial ABC toxins revealed in molecular detail

*Christos Gatsoqiannis*¹, *Dominic Meusch*¹, *Alexander E Lang*², *Klaus Aktories*², *Stefan Raunser*¹

¹Department of Structural Biochemistry, Max Planck Institute of Molecular Physiology, Dortmund, Germany; ²Institut für

Experimentelle und Klinische Pharmakologie und Toxikologie,
Albert-Ludwigs-Universität, Freiburg, Germany
Correspondence: christos.gatsogiannis@mpi-dortmund.mpg.de

Tc toxin complexes are dominant secreted virulence factors of many pathogenic bacteria such as the Far East scarlet-like fever pathogen *Yersinia pseudotuberculosis*, the plague pathogen *Yersinia pestis* and the insect pathogenic *Photorhabdus luminescens*. They are typically composed of TcA, TcB and TcC subunits that are only biologically active as tripartite complexes. TcB and TcC together form a closed cage, that encapsulates and sequesters the cytotoxic, C-terminal region of the C protein like the shell of an egg. However, little is known about the translocation of this toxic component into the cell by the TcA component.

Here we show that TcA in *Photorhabdus luminescens* (TcdA1) forms a transmembrane pore and report its structure in the prepore and pore state determined by cryoelectron microscopy and x-ray crystallography. We find that the TcdA1 prepore assembles as a pentamer forming an α -helical, vuvuzela-shaped channel less than 1.5 nm in diameter surrounded by a large outer shell. Membrane insertion is triggered not only at low pH as expected, but also at high pH, most probably explaining Tc action directly through the midgut of insects. Comparisons with structures of the TcdA1 pore inserted into a membrane and in complex with TcdB2 and TccC3 reveal large conformational changes during membrane insertion, suggesting a novel syringe-like mechanism of protein translocation driven by an entropic spring.

Our results allow us for the first time to understand key steps of infections involving ABC toxins at molecular level and shed new light on the interaction of the bacterial pathogens with their hosts.

SYMPOSIUM PAPER Wednesday 11:30 **91**

***Caenorhabditis elegans nck-1* plays a distinct and specific role in defense against bacterial pore-forming toxins**

Anand Sitaram, Yunqiang Yin, Raffi V. Aroian

University of Massachusetts Medical School, Worcester, MA, USA
Correspondence: Raffi.Aroian@umassmed.edu

The largest class of bacterial protein toxins is the pore-forming toxins (PFTs), produced by a variety of human pathogens. The proteins oligomerize on and perforate the host cell membrane, ultimately leading to cell lysis and organismal death. In many cases, pathogens deleted for PFTs become avirulent, revealing the importance of understanding the biology of these toxins. At sublytic PFT doses, target cells activate conserved innate defenses, many of which our lab has characterized using *Caenorhabditis elegans* and the nematocidal PFT Cry5B, one of a family of PFTs produced by *Bacillus thuringiensis*. After performing an RNAi screen for genes whose inactivation makes animals hypersensitive to Cry5B, we focused on further investigating the function of *nck-1*, a homolog of mammalian Nck. Loss of *nck-1* activity led to an unusually specific sensitization to Cry5B and other PFTs but not to other types of stress. *nck-1* function in PFT defense also appears to be distinct from several other defense pathways that have been previously characterized. We continue to explore the important and new role that *nck-1* plays in host cellular defense against pore-forming toxins using a combination of genetic, genomic, and cell biological techniques.

SYMPOSIUM PAPER Wednesday 11:50 **92**

The cell biology of *Wolbachia*-filarial nematode interactions and the dark side of symbiosis

F. Landmann^{1,2}, J. M. Foster³, M. L. Michalski⁴,
B. E. Slatko⁵, W. Sullivan¹

¹Department of Molecular, Cell and Developmental Biology, Sinsheimer Labs, University of California, Santa Cruz, California, United States of America; ²Centre de Recherche de Biochimie Macromoléculaire, CNRS, Montpellier, France; ³Molecular Parasitology, New England Biolabs, Ipswich, Massachusetts, United States of America; ⁴Department of Biology and Microbiology, University of Wisconsin Oshkosh, Oshkosh, Wisconsin, United States of America

Correspondence: sullivan@biology.ucsc.edu

Filarial nematodes maintain a mutualistic relationship with the bacterial endosymbiont *Wolbachia*, however the molecular and cellular basis of this relationship remains unclear. In embryos derived from *B. mayali*, the causative agent of lymphatic filariasis, we found the normal posterior localization of *Wolbachia* relies on cortical microtubules and the motor protein Dynein. In addition, *Wolbachia* also required Par-1 and Par-3 polarity determinants for normal concentration at the posterior cortex. Surprisingly, we also found the converse: removal of *Wolbachia* resulted in distinct *par* mutant-like defects in anterior-posterior embryonic axis determination. Thus, *Wolbachia* is essential for A-P axis determination, a key event in early embryogenesis. In addition, previous studies demonstrated that removal of *Wolbachia* from adult nematodes resulted in extensive apoptosis, even in tissues and cells not infected with *Wolbachia*. These findings indicate that *Wolbachia* have functionally integrated into core developmental and cellular processes such that it is essential for filarial nematode reproduction and survival. It may be that the nematode host outsourced core cellular processes to this invading endosymbiont, making a normally parasitic relationship essential for survival of the nematode.

SYMPOSIUM PAPER Wednesday 12:10 **93**

***Photorhabdus*: Light without heat?**

Alexia Hapeshi¹, Geraldine Mulley², Joe Healey¹, Andrew Millard,
Nick Waterfield¹

¹Warwick Medical School, Warwick University, Gibbet Hill, Coventry CV1 7AL, UK; ²School of Biological Sciences, University of Reading, Whiteknights, Reading, RG6 6AJ, UK
Correspondence: N.R.Waterfield@warwick.ac.uk

Most Enterobacteriaceae have a broad range of temperature tolerance, growing up to and beyond 37°C. The genus *Photorhabdus* are somewhat unusual in that the majority of strains cannot grow and reproduce much above 34°C. This thermal intolerance has implications for the potential host range of these pathogens, limiting them to poikilothermic insects. The exceptions to this are the clinical isolates belonging to the *P. asymbiotica* species, which necessarily need to be able to grow at 37°C and can infect insects or humans. Interestingly certain *P. luminescens* strains can also grow at 37°C, although no members of this species have yet been associated with mammalian infection. We are trying to understand what molecular adaptations can allow members of this ubiquitous reservoir of insect pathogens to evolve human pathogenicity. We have been addressing this question using two complementary approaches. Firstly we have used integrated post-genomic analysis techniques to assess the genotypic and phenotypic behavior of *P. asymbiotica* isolates at 28°C and 37°C, representing insect and human host temperatures respectively. Secondly we have employed laboratory selection to isolate temperature tolerant mutants of *P. luminescens*^{TT01}, a strain usually restricted to 34°C. Our analysis of these mutants has identified a gene which prevents this strain from growing at 37°C. We are currently working to understand

the mechanism and evolutionary implications of this. Finally, to put our analyses into context we have performed whole genome sequencing of a range of *P. asymbiotica* isolates from different continents to derive a whole genome phylogeny of these human pathogenic strains.

CONTRIBUTED PAPERS Wednesday 10:30 – 12:30

Fungi 1

CONTRIBUTED PAPER Wednesday 10:30 **94**

RNA-seq analysis reveals the potential antioxidant pathways regulated by multiprotein bridging factor 1 (*BbMBF1*) in the fungal entomopathogen *Beauveria bassiana*

Xin-Ling Chu, Ming-Guang Feng, Sheng-Hua Ying

College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, 310058, China

Correspondence: yingsh@zju.edu.cn

Oxidation resistance is crucial to environmental fitness and potency of the fungal entomopathogen *Beauveria bassiana*. Multiprotein bridging factor 1, an evolutionarily conserved transcriptional cofactor, plays an important role in development and stress tolerance of eukaryotic organisms. It has been established that the *B. bassiana* homolog (*BbMBF1*) contributes to the fungal response to menadione stress under lower nutritional conditions. Present study indicated that rich nutrient reduces the fungal sensitivity to menadione stress, but further confirmed that the *BbMBF1* is also required for the fungal tolerance to oxidation under rich nutritional conditions. To explore the down-stream targets regulated by *BbMBF1* under menadione stress, a comparative transcriptome was performed on the wild type and the $\Delta BbMBF1$ mutant strains. Transcriptomic analysis showed that the oxidation-response genes regulated by *BbMBF1* were significantly enriched in the functional catalogs of metabolism, cell rescue, and transportation. Moreover, bioinformatic analysis predicted a putative motif which distributed mainly over the promoters of genes associated with metabolism and detoxification. Conclusively, these results indicate that *BbMBF1* contributes to *B. bassiana* response to oxidative stress by mediating the potential transcription factors regulating pathway of metabolism and detoxification. These findings highlight the potential antioxidant strategies in the fungal entomopathogens, and provide the new clues/targets for improving potency of entomopathogenic fungi by molecular manipulation

CONTRIBUTED PAPER Wednesday 10:45 **95-STU**

Unveiling a link of the Fus3 pathway to the biological control potential of *Beauveria bassiana*

Zhi-kang Wang, Ming-Guang Feng

Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, 310058, China

Correspondence: mgfeng@zju.edu.cn

The Fus3 pathway comprises the cascaded protein kinases Fus3 (MAPK), Ste7 (MAPKK) and Ste11 (MAPKKK) in filamentous fungi and has not been fully explored in fungal insect pathogens. In this study, we characterized the cascaded proteins and upstream Ste50 in *Beauveria bassiana*. Single-deletion mutants of four genes coding the proteins grew slower and conidiated less than wild-type, and lost whole virulence due to an incapability their penetrating through insect cuticle. Cell cycle analysis of their unicellular blastospores revealed significantly longer G₂/M

transition, which was evident with weakened phosphorylation signal of cyclin-dependent kinase 1 (Cdk1) required for normal cell cycle. In addition, the four deletion mutants showed different degrees of increased sensitivities to nutritional, fungicidal, thermal, and UV-B irradiative stresses. The results highlight a link of the Fus3 pathway to the biological control potential of *B. bassiana*.

CONTRIBUTED PAPER Wednesday 11:00 **96-STU**

Contributions of monothiol glutaredoxin and glutathione reductase to antioxidant capability and biological control potential of *Beauveria bassiana*

Long-Bin Zhang, Li Tang, Ming-Guang Feng

Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, 310058, China

Correspondence: mgfeng@zju.edu.cn

Glutaredoxins (Grx) involved in cellular thiol-disulfide redox system function in conjunction with thioredoxins but are poorly understood in fungal filamentous insect pathogens. *Beauveria bassiana* harbors five Grx homologs (Grx1–5) and one glutathione reductase (Glr), which are classified to the distinct clades dithiol Grx, monothiol Grx and Grx-like protein respectively. Deletion of a *grx* homolog of *glr* resulted in transcriptional compensation of other partners despite undeletable *grx4*. Deletion of *grx3* or *glr* reduced significantly the activities of the antioxidant enzymes superoxide dismutases (SODs), glutathione-peroxidase and oxidized/reduced glutathione. Consequently, the Δglr and $\Delta grx3$ mutants became significantly more sensitive to two oxidants (H₂O₂ and menadione) and two thiol oxidizing agents than wild-type during conidial germination and colony growth. The two mutants showed remarkable defects in conidiation, conidial germination, conidial thermotolerance, conidial UV-B resistance and virulence. In addition, intracellular iron homeostasis was biased in the Δglr and four Δgrx mutants. Among the characterized proteins, Grx3 and Glr were proven to play much more important roles in regulating cellular redox homeostasis and antioxidant responses than other Grx homologs except Grx4, thereby contributing significantly to the biological control potential of *B. bassiana*.

CONTRIBUTED PAPER Wednesday 11:15 **97-STU**

The Pal pathway regulates growth, conidiation, acidification, osmotic stress and virulence in *Beauveria bassiana*

Jing Zhu, Ming-Guang Feng

Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, 310058, China

Correspondence: mgfeng@zju.edu.cn

Adaption to environmental pH is critical for fungal survival and host infection and regulated by the Pal pathway in filamentous fungi. The Pal pathway of *Beauveria bassiana* comprises PacC and PalA/B/C/F/H/I homologs. Alkaline and stressful conditions upregulated much more transcriptional expression of *pacC* than the *pal* genes in wild-type. Single-deletion mutants of these genes except $\Delta pall$ showed growth defects in an alkaline medium (pH 9.0) but grew as well as wild-type in an acidic medium (pH 3.0). For all the deletion mutants, conidiation level was increased under acidic and neutral conditions but slightly decreased under alkaline conditions except for an increase in $\Delta pall$, followed by increased size and density of their conidia. Extra- and intracellular production of organic acids, such as lactic acid, oxalic acid and citric acid, was up- or -down-regulated by different degrees and hence resulted in much delayed acidification in their liquid cultures, accompanied with transcriptional up-regulation of

several genes involved in acid biosynthesis. Additionally, the deletion mutants were sensitive to osmotic stress of NaCl under alkaline conditions and less virulent to a susceptible insect. Our results indicate a significance of the Pal pathway for growth, conidiation, osmotolerance and virulence of *B. bassiana*.

CONTRIBUTED PAPER Wednesday 11:30 **98-STU**

Regulative role of a novel Ras GTPase (Ras3) in conidiation, stress responses and virulence and its involvement in the HOG pathway of *Beauveria bassiana*

Yi Guan, Ding-Yi Wang, Ming-Guang Feng

Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, 310058, China

Correspondence: mgfeng@zju.edu.cn

Two Ras GTPases (Ras1 and Ras2) are well known to regulate antagonistically or cooperatively various cellular events in many fungi, contrasting to a novel Ras homolog (Ras3) not characterized in filamentous fungi. Ras3 in *Beauveria bassiana* has five domains and two GTP/GDP switches typical for the Ras family and was proven to localize in plasma membrane in this study. Deletion of *ras3* altered greatly temporal transcription patterns of *ras1* instead of *ras2*. Compared with wild-type, $\Delta ras3$ grew significantly faster in a rich medium but slower in some minimal media, and produced far fewer conidia with impaired quality, which was evident with slower germination, attenuated virulence, reduced thermotolerance and decreased UV-B resistance. Moreover, $\Delta ras3$ was much more sensitive to the oxidative stress of menadione than of H₂O₂ and to the stress of high osmolarity than of cell wall perturbation during growth. All the phenotypic changes were restored by *ras3* complementation. The high sensitivity of $\Delta ras3$ to menadione was concurrent with depressed gene transcripts and reduced total activity of superoxide dismutases. Intriguingly, the high osmosensitivity was concurrent not only with drastically depressed transcripts of a critical transcription factor (Msn2) and most signaling proteins in the high-osmolarity-glycerol (HOG) pathway of $\Delta ras3$ but with largely weakened phosphorylation signal of Hog1 hallmarking the pathway. Overall, Ras3 is involved in the Hog1 pathway required for cellular osmoregulation and hence can mediate positively conidiation, germination, multi-stress tolerance and virulence linked to the biological control potential of *B. bassiana*.

CONTRIBUTED PAPER Wednesday 11:45 **99-STU**

A genome wide association study of resistance to *Metarhizium anisopliae*

Jonathan B. Wang, Hsiao-Ling Lu, Raymond J. St. Leger

Entomology Department, University of Maryland, College Park, MD, USA

Correspondence: jonwang@umd.edu

In a sister study to this one we screened more than 2,500 mutations for their effects on disease resistance. A complementary approach to mutagenesis is to identify loci at which alleles with more subtle effects segregate in natural populations. Single nucleotide polymorphisms (SNPs), insertions, and deletions in a natural population of flies are mutations that have survived the filter of natural selection and can be tested via genome-wide association (GWA) for effects on genetic variation in resistance. Here we used 203 fly lines from the Drosophila Genetic Reference Panel (DGRP) to identify SNPs associated with natural variation to disease resistance. Using topical infections with *Metarhizium anisopliae* (Ma549), we calculated LT50's, fungal loads, latent period (interval between infection and sporulation), and sporulation capacity. All parameters were genetically variable

among the DGRP lines and LT50s were sexually dimorphic. We identified many SNPs in novel loci that are potentially associated with natural variation in disease resistance, as well as SNPs within genes previously known to affect resistance. Many of these loci are known to interact physically and/or genetically, enabling us to place them in candidate genetic networks. Several of the candidate genes have human homologues that were identified in studies of human disease, suggesting that genes affecting variation in susceptibility are conserved across species.

CONTRIBUTED PAPER Wednesday 12:00 **100-STU**

GeoChip analysis of the soil microbial community in turf and winter wheat treated with genetically modified *Metarhizium*

Brian Lovett, Raymond St. Leger

University of Maryland, College Park

Correspondence: lovettbr@umd.edu

The availability of genome sequences and robust technologies have allowed production of genetically manipulated *Metarhizium* strains that could represent a major new arsenal for combating pest insects and enhancing plant growth. However, for these tools to be used safely, we need thorough understanding of the impact they would have in a new environment. This research examines how intensive deployment of transgenic *M. robertsii* impacts microbial communities in the soil root interphase (a preferred habitat of *Metarhizium*) in natural (turf) and agricultural (winter wheat) field sites. In our investigation, we employed a high-throughput metagenomic tool (GeoChip 4.6) that is able to assess all functionally known geochemical, ecological, and environmental processes including N, C, S, and P cycling, metal reduction and resistance, and organic xenobiotic degradation. Our study provides a comprehensive analysis into how and to what extent applied *Metarhizium robertsii* can alter community structure and key functional roles. As well, this study provides a unique opportunity to use a global-scale methodology to compare the activity of the microbial communities of agricultural versus natural soil systems and of bulk soil versus the rhizosphere.

CONTRIBUTED PAPER Wednesday 12:15 **101**

Diversity and distribution of *Metarhizium flavoviride* in agroecosystems: An overlooked entomopathogenic fungus?

Chad A. Keyser, Henrik H. De Fine Licht, Bernhardt M.

Steinwender, Nicolai V. Meyling

Department of Plant and Environmental Sciences, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg, Denmark

Correspondence: nvm@plen.ku.dk

Knowledge of the natural occurrence and community structure of entomopathogenic fungi is important to understand their ecological role. Within the genus *Metarhizium*, the species *M. flavoviride* has received little attention and intra-specific diversity among isolate collections has never been assessed. We found *M. flavoviride* to be abundant among *Metarhizium* spp. isolates obtained from roots and root-associated soil of winter wheat, winter oilseed rape, and neighboring uncultivated pastures at three geographically separated agricultural fields in Denmark, and we evaluated the molecular diversity to resolve the potential population structure of *M. flavoviride*. Of the 132 *Metarhizium* isolates obtained, morphological data and DNA sequencing revealed that 118 belonged to *M. flavoviride*, 13 to *M. brunneum* and one to *M. majus*. Further characterization of intraspecific variability within *M. flavoviride* was achieved by using amplified fragment length polymorphism (AFLP) to evaluate diversity and potential crop and/or area associations. A high level of diversity among the *M. flavoviride* isolates was observed. However, no

population structure in the form of significant haplotype groupings or habitat associations could be determined among the 118 analyzed *M. flavoviride* isolates. The ecological significance and natural distribution of *M. flavoviride* will be discussed by including earlier observations in Denmark.

CONTRIBUTED PAPERS Wednesday 10:30-12:30

Viruses 3

CONTRIBUTED PAPER Wednesday 10:30 **102**

Insecticidal parameters for the baculovirus infecting *Agrotis ipsilon* neonates

Robert W. Behle

USDA-ARS-NCAUR, Crop Bioprotection Research Unit, Peoria, Illinois, USA

Correspondence: robert.behle@ars.usda.gov

The recently discovered multi-nuclear polyhedrosis virus capable of infecting larvae of the *Agrotis ipsilon* (Hufnagel) (black cutworm) has the potential to be developed as a biological insecticide for controlling this polyphagous pest of crops. High toxicity and short lethal time are necessary characteristics for the virus to provide effective control and prevent plant damage, but are not interchangeable as assessments for treatment evaluations. Toxicity (LC₅₀) and lethal time (LT₅₀) were determined using a droplet-feeding laboratory assay with neonates. Probit analysis calculated the LC₅₀ to be 1.2×10^5 occlusion bodies (OB) ml⁻¹. Lethal times were determined for larvae killed by virus after exposure to one of four virus dosages representing the estimated LC₃₀, LC₅₀, LC₇₀, and LC₉₀ concentrations (0.51×10^5 , 1.2×10^5 , 3.0×10^5 , and 10.7×10^5 OB ml⁻¹), with LT₅₀ values of 67.1, 65.3, 64.4, and 61.5 hours, respectively. Thus, LT₅₀ values for larvae exposed as neonates decreased significantly, although only slightly with increasing virus concentration. Altering experimental conditions such as the type of experiment (laboratory versus field), type of exposure (acute versus chronic), and larval size at the time of exposure will likely impact values for toxicity and lethal time. The low LC₅₀ and short LT₅₀ reported here demonstrate the suitability of this agent for further consideration as a biopesticide for control of *A. ipsilon*, although additional studies are necessary to select appropriate parameters to measure for future research to support product development.

CONTRIBUTED PAPER Wednesday 10:45 **103**

Haemocytes from *Crassostrea gigas* and OshV-1: A promising *in vitro* model to study host/virus interactions

Benjamin Morga¹, Nicole Faury¹, Tristan Renault²

¹Ifremer (Institut Français de Recherche pour l'Exploitation de la Mer) Laboratoire de Génétique et Pathologie des Mollusques Marins, La Tremblade, France; ²Ifremer Département Ressources Biologiques et Environnement, Nantes, France

Correspondence: benjamin.morga@ifremer.fr

Since 2008, mass mortality outbreaks associated with OshV-1 detection are reported in *Crassostrea gigas* spat and juveniles in several countries. Some recent studies reported information on viral replication during an experimental infection. Viral RNA detection was noticed in spat mantle 4h post virus suspension injection. Moreover, an *in situ* hybridization approach showed that OshV-1 mRNAs were mainly present in the connective tissue of gills, mantle, adductor muscle, digestive gland and gonads following the injection of the virus suspension in the muscle. Consequently, one hypothesis putted forward is that the virus

could be transported by the haemolymph. In oyster haemolymph contains immune cells, the haemocytes. In this context, is the virus transported in the haemolymph or is it able to initiate a replication in haemocytes? No marine mollusc cell lines are available. In the present study, we have thus collected haemocytes from the adductor muscle of *C. gigas* spat and put them *in vitro* in contact with a viral suspension. Results showed that viral RNA were detectable one hour after contact and the number of virus transcripts increased across the time of contact in association with an increase of viral DNA detection. These results suggested that the virus is able to initiate replication rapidly inside haemocytes maintained *in vitro*. These *in vitro* trials were also used to carry out a dual transcriptomic study. We analysed concomitantly the expression of some host immune genes and the expression of viral genes. Results showed an up regulation of oyster genes currently studied in this model during an OshV-1 infection. All the results suggest that the *in vitro* model based on the use of haemocytes can be a valuable model opening new perspectives host – pathogen interactions.

CONTRIBUTED PAPER Wednesday 11:00 **104-STU**

Comparison of viral growth characteristic of two nucleopolyhedroviruses isolated from the genus *Adoxophyes* *Yasumasa Saito^{1,2}, Yasuhisa Kunimi¹, Maki N. Inoue¹, Madoka Nakai¹*

¹Laboratory of Biological Control, United Graduate School of Agricultural Science, Tokyo University of Agriculture and Technology, 3-5-8 Saiwai-cho, Fuchu, Tokyo 183-8509, Japan;

²Japan Society for the Promotion of Science

Presenting: saitoyassu@gmail.com

Correspondence: madoka@cc.tuat.ac.jp

The smaller tea tortrix, *Adoxophyes honmai* (Lepidoptera: Tortricidae), is susceptible to two nucleopolyhedroviruses (NPVs), *A. honmai* NPV (AdhoNPV) and *A. orana* NPV (AdorNPV), which are genetically closely related but differ in killing speed. AdhoNPV is slow-killing and the infected host is always killed in the final instar regardless of the infection timing, whereas AdorNPV is fast-killing and it kills the host in the same instar or after one molting. In this study, we investigated whether killing speed of both of viruses are related to viral growth speed. Second and 4th instars of *A. honmai* were orally inoculated with >LC₉₅ of AdhoNPV or AdorNPV. Then, each inoculated host was collected every 24h and larval weight and viral DNA were quantified. Viral DNA was measured by qPCR and the growth curve was fit to “modified Gompertz model” which consists of three parameters as maximum concentration of viral DNA (A), maximum rate of viral increase (μ_m) and time lag. Mean lethal time of 2nd and 4th inoculation was 15 and 7 days, and 10 and 8 days for AdhoNPV- and AdorNPV-inoculated host, respectively. For 2nd instar inoculation, “A” of AdhoNPV-infected was significantly higher than that of AdorNPV-infected, whereas “μ_m” of AdorNPV-infected was significantly higher than that of AdhoNPV-infected. On the other hand, there was no significant difference of both parameters between AdhoNPV and AdorNPV at 4th instar inoculation. In conclusion, the higher viral growth speed (μ_m) can be attributed to faster killing speed of AdorNPV as compared to AdhoNPV at 2nd instar inoculation.

CONTRIBUTED PAPER Wednesday 11:15 **105-STU**

A novel type of resistance of the codling moth against *Cydia pomonella* granulovirus shows two different resistance mechanisms

Annette J. Sauer, Manuela M. Gebhardt, Eva Fritsch, Karin Undorf-Spahn, Johannes A. Jehle

Institute for Biological Control, Federal Research Center for Cultivated Plants, Julius Kühn Institute, Darmstadt, Germany
Correspondence: Annette.Sauer@iki.bund.de

Control of codling moth (CM, *Cydia pomonella*) in pome fruit production is successfully achieved by applying *Cydia pomonella* granulovirus (CpGV, *Baculoviridae*). Since 2005, CM populations resistant to the widely used isolate CpGV-M have been found in about 40 orchards in different European countries. The resistance allele of most of these populations is assumed to be dominant and located on the sex-chromosome Z. Most populations can be controlled by resistance-breaking isolates, such as CpGV-S. However, one population in Germany showed resistance to both CpGV-M and the resistance-breaking isolate CpGV-S. In order to elucidate this unusual type of resistance, successive mass crossings of this population were carried out under virus pressure to establish a genetically homogenous resistant CM strain, called CpR5M. Subsequent reciprocal crossing experiments with CpR5M and a susceptible laboratory CM strain (CpS) followed by bioassays revealed a dominant but autosomal inheritance. Whereas CpR5M was also resistant to mixed infections with CpGV-M and CpGV-S, CpR5M was fully susceptible to a recombinant CpGV-M carrying the *pe38* of CpGV-S. These results suggest that two independent resistance mechanisms, one directed against *pe38* of CpGV-M and a second one directed against an unknown factor of CpGV-S but not present in CpGV-M are involved in this type of resistance. Because a recombinant genome of CpGV-M and CpGV-S but not a mixture of CpGV-M and CpGV-S can overcome this novel type of resistance, it is further concluded that resistance to CpGV-S is most likely directed to an extracellular receptor, whereas resistance to CpGV-M is systemically directed via *pe38*.

CONTRIBUTED PAPER Wednesday 11:30 **106-STU**

Nucleopolyhedrovirus and microsporidia in winter moth (*Operophtera brumata*) and bruce spanworm (*O. bruceata*) populations in the northeastern U.S.

[Hannah J. Broadley](#)¹, [Joseph S. Elkinton](#)¹, [John P. Burand](#)², [Matt Boucher](#)², [Leellen F. Solter](#)³

¹Organismic and Evolutionary Biology, University of Massachusetts, Amherst, MA, U.S.A.; ²Department of Microbiology, University of Massachusetts, Amherst, MA, U.S.A.; ³Department of Natural Resources and Environmental Sciences.

University of Illinois, Champaign, IL, USA
Correspondence: hbroadley@cns.umass.edu

Winter moth (WM, *Operophtera brumata*), a polyphagous geometrid, was accidentally introduced to the northeastern United States from Europe in the 1990s. Since its introduction, WM has exhibited population outbreaks. However, its native congener, Bruce spanworm (BSW, *O. bruceata*), rarely outbreaks. We propose that this difference in population dynamics exists because BSW experiences different pathogens, which may exist at a higher prevalence or be more virulent. Field collected WM and BSW larvae were reared in the lab and percent mortality was noted for 2013 and 2014. Cadavers were examined microscopically for evidence of nucleopolyhedrovirus (NPV) and microsporidia (possibly *Nosema* sp.) infections. For both species, we amplified the polyhedron and p74 gene from NPV isolates and compared them using phylogenetic analysis. To test for cross infection, WM eggs were surface sterilized, induced to hatch, and fed diet infected with BSW NPV. Moribund larvae were analyzed for NPV. Across years, BSW larvae suffered higher mortality (~26%) than did WM (~3.2%). BSW had higher rates of microsporidian infection (23x higher), while WM experienced higher rates of NPV (3.3x higher). The BSW polyhedron gene

sequence was only 82% identical to WM, while the p74 sequence was most closely related to, but distinct from, WM. This indicates that NPV infecting these species are different but only recently diverged. In cross-infection trials, BSW NPV did not infect WM larvae. In conclusion, WM and BSW experience different pathogens at different rates. Understanding the activity of epizootics in these species may provide insight into biological control agents for WM.

CONTRIBUTED PAPER Wednesday 11:45 **107-STU**

How climate and host behaviour influence nucleopolyhedrovirus infection dynamics in the western tent caterpillar

[Paul R. MacDonald](#)¹, [Judith H. Myers](#)², [Jenny S. Cory](#)¹

¹Department of Biological Sciences, Simon Fraser University, Burnaby, BC, Canada

²Department of Zoology, University of British Columbia, Vancouver, BC, Canada

Correspondence: pmacdona@sfu.ca

Western tent caterpillar (WTC) populations display cyclical dynamics with 8-11 year periodicity in southwestern B.C. Long-term population data show that nucleopolyhedrovirus (NPV) is an integral component of these dynamics, with high incidence of viral mortality coinciding with substantial population decline. Our research involves two areas of NPV transmission that remain unclear. Firstly, how climate influences NPV transmission and secondly, how host behaviour contributes to transmission and the eventual formation of an epizootic. Using historical population and weather data we have shown a correlation between warm springtime temperatures and heightened levels of NPV infection. We conducted a series of laboratory and field-based experiments that suggest possible mechanisms for this observation. Additionally, we have conducted experiments that reveal whether infected hosts differ from uninfected hosts in their behaviour and discuss how these differences could facilitate NPV transmission at the population scale. We conclude by discussing how empirical evidence and theoretical modelling may be used to predict how WTC population dynamics behave under various climate and behavioural scenarios.

CONTRIBUTED PAPER Wednesday 12:00 **108-STU**

The specialist baculovirus SeMNPV induces light-dependent tree-top disease in *Spodoptera exigua* caterpillars facilitated by the viral *egt* gene

[Yue Han](#)¹, [Stineke van Houte](#)², [Monique M. van Oers](#)¹, [Vera I. D. Ros](#)¹

¹Laboratory of Virology, Wageningen University, Droevendaalsesteeg 1, 6708 PB, Wageningen, the Netherlands
²Centre for Ecology and Conservation, Biosciences, University of Exeter, Penryn, Cornwall, United Kingdom

Correspondence: yue.han@wur.nl

Many parasites enhance their transmission by manipulating host behaviour. One example concerns baculoviruses that induce hyperactivity and tree-top disease (i.e. climbing to elevated positions prior to death) in their caterpillar hosts. Little is known about the underlying mechanisms of such parasite-induced behavioural changes. Here, we studied tree-top disease in *Spodoptera exigua* caterpillars induced by the specialist baculovirus *S. exigua* multiple nucleopolyhedrovirus (SeMNPV). We found that SeMNPV induced positive phototactic climbing behaviour prior to death in *S. exigua* larvae. In addition, we showed that larvae infected with a mutant virus lacking the ecdysteroid UDP-glucosyltransferase (*egt*) gene showed a shorter time to death and died before the onset of phototaxis. Moreover,

deletion of either the open reading frame or the ATG start codon of the *egt* gene prevented tree-top disease, indicating that the EGT protein is required for tree-top disease. We hypothesize that EGT facilitates the phototactic climbing behaviour via prolonging the larval time to death.

CONTRIBUTED PAPER Wednesday 12:15 **109**

Baculovirus-induced tree-top disease revisited: The role of the *egt* gene

Vera I. D. Ros¹, Yue Han¹, Stineke van Houte^{1,2}, Monique M. van Oers

¹Laboratory of Virology, Wageningen University, Droevendaalsesteeg 1, 6708 PB, Wageningen, the Netherlands; ²Centre for Ecology and Conservation, Biosciences, University of Exeter, Penryn, Cornwall, United Kingdom
Correspondence: vera.ros@wur.nl

Baculovirus-infected caterpillars climb to elevated positions prior to death, a phenomenon known as ‘tree-top disease’. The past years, several studies have focused on the underlying mechanism, considering a range of baculovirus-host systems. Particularly, the role of the viral ecdysteroid UDP-glucosyltransferase (*egt*) gene has received attention. In some virus-host interactions *egt* seems to induce tree-top disease, while in other systems *egt* has no apparent role in tree-top disease or has an indirect role, through affecting the time to death and/or moulting-related climbing behaviour. Here, we review the current knowledge on the mechanism of tree-top disease, including an overview on the role of the *egt* gene.

SYMPOSIUM Wednesday 14:00 – 16:00

**Endophytic Entomopathogenic Fungi:
“Pro-biotic” Microbial Associates of Plants?**

SYMPOSIUM PAPER Wednesday 14:00 **110**

Endophytic entomopathogenic fungi as “plant probiotics”: An important tool in protecting and promoting plant health?

Chad A. Keyser, Donald W. Roberts

Department of Biology, Utah State University, Logan Utah, USA
Correspondence: chadaltkeyser@gmail.com

Élie Metchnikoff is well-known among many Society for Invertebrate Pathology members as the discoverer of the first isolate of the entomopathogenic fungal genus *Metarhizium*. He is also credited, however, with being the first to propose ingesting certain microorganisms to improve health in humans – a phenomenon now known as “probiotics”. Probiotics are defined as: *live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host*. Currently the term “probiotics” is only used to refer to interaction between beneficial microbes and human/animal hosts; however, in a biological control context, some rhizosphere-competent and -endophytic microbes behave “probiotic-like” in that they promote plant health. The primary modes of action for probiotics in mammals include: competition for nutrients, competition for adhesion sites, direct antagonism, and immune stimulation. Research findings within the past decade indicate that these same modes of action are often seen following application of microbes on plants to control plant pests or pathogens. As will be discussed in this symposium, it is abundantly clear that entomopathogenic fungi are both endophytic and interact beneficially with plants. Viewing entomopathogenic organisms as potentially playing a “probiotics-

like” role for a host plant emphasizes their importance in both plant protection and plant-health promotion.

SYMPOSIUM PAPER Wednesday 14:30 **111**

Entomopathogenic fungi as endophytes: Interactions with host plants and herbivores

Stefan Vidal¹, Anant Patel²

¹Georg-August-University Goettingen, Department of Crop Sciences, Goettingen, Germany; ²University of Applied Sciences, Department of Engineering and Mathematics, Bielefeld, Germany
Correspondence: svidal@gwdg.de

There is growing evidence that most, if not all, entomopathogenic fungi are able to colonize plant tissues as fungal endophytes in most, if not all plant species. Depending on the specific organisms involved these interactions may be beneficial to both the plant and the fungus, neutral or even antagonistic. Data so far published in this regard mainly refer to *Beauveria bassiana*, but *Metarhizium anisopliae* and *Lecanicillium lecanii* have also been shown to endophytically colonize plant tissues. Given the prevailing problems in using entomopathogenic fungi as biocontrol agents under field conditions it sounds like a good idea to use these fungi as endophytes, which would allow protecting the crop plants from the seedling stage to harvest. However, several open questions and constraints need to be addressed before these organisms can be fully implemented as plant protection agents. Data will be presented addressing the following issues:

- Do plants respond to the inoculation by entomopathogenic endophytes with changes in physical or physiological parameters?
- Are data available corroborating that re-growing plant parts are systemically colonized following an inoculation via the seeds or the roots?
- Is there sufficient evidence that an inoculation of a plant results in higher mortality of herbivores feeding on these plants?
- Are plants colonized by these endophytes less attractive for herbivores and do volatile pattern contribute to plant-herbivore interactions?
- Are there technical strategies available to increase the efficacy of entomopathogens as endophytic biocontrol agents?

Results addressing these questions are discussed in the context of improving herbivore pest control options.

SYMPOSIUM PAPER Wednesday 15:00 **112**

Trading insect nitrogen for photosynthate: Carbon translocation from a plant to an insect pathogenic, endophytic fungus

Michael J. Bidochka, Scott W. Behie

Department of Biological Sciences, Brock University, St. Catharines, ON Canada
Correspondence: bidochka@brocku.ca

Many vascular plants are able to form close symbiotic associations with endophytic fungi. *Metarhizium* is a common soil fungus that is both a plant endophyte and an insect pathogen. Previously we have shown that the endophytic capability and insect pathogenicity of *Metarhizium* are coupled to provide an active method of nitrogen transfer to a host plant via fungal mycelia. We speculated that in a reciprocal exchange for insect-derived nitrogen, the plant provided photosynthate to the fungus. We used ¹³CO₂ in airtight plant growth chambers to track the incorporation of ¹³C into plant (haricot bean; *Phaseolus vulgaris*) carbohydrates and the subsequent translocation of ¹³C

into fungal-specific carbohydrates (trehalose and chitin) in the root/endophytic fungal complex. The amount of ^{13}C present in root-associated fungal biomass was determined over a 21 day period by sequentially extracting trehalose and N-acetylglucosamine (GlcNAc) from roots and analyzing for ^{13}C -trehalose and ^{13}C -GlcNAc using nuclear magnetic resonance (NMR) spectroscopy. The translocation of photosynthate to endophytic *Metarhizium* occurred in the absence of a host insect (as a nitrogen source) in the soil; however, in the presence of an insect host we observed a significant increase in plant-derived photosynthate in fungal-specific carbohydrates. These findings are evidence that the host plant is trading photosynthate for insect-derived nitrogen in this tripartite symbiotic interaction that involves a plant, an endophytic insect pathogenic fungus and soil insects.

SYMPOSIUM PAPER Wednesday 15:30 **113**

***Metarhizium* as a multifactorial plant growth promoter**

Raymond J. St. Leger

Department of Entomology, University of Maryland, Maryland, USA, 20742

Correspondence: stleger@umd.edu

Metarhizium spp are amongst the most abundant fungi isolated from soils world-wide, under various environmental conditions, with sparse nutrients and in the presence of compounds lethal to other fungi. Several labs have shown that the *Metarhizium* fungal species used for biocontrol of insect pests are capable of interacting with plant roots directly, behaving as symbiotic microorganisms. Many narrow host range *Metarhizium* strains germinate poorly on plant roots and have no impact on root or shoot growth, whereas strains that grow well on roots without increasing plant growth usually have plant growth promoting relations. Root colonizing *Metarhizium* strains promote plant growth by mechanisms that include killing insects, making nutrients available, increasing stress resistance and producing growth promoting metabolites. I will discuss how a combination of mass spectrometry, mutant analysis, easily identified marker genes and genomic tools for identifying genetic changes has provided knowledge of how *Metarhizium* persists on plant roots and its modes of promoting plant growth. Field trials have confirmed that the ability to adhere to root surfaces plays an important part in maintaining *Metarhizium* population size, irrespective of the presence of insects, and that some strains are multifactorial plant growth promoters.

CONTRIBUTED PAPERS Wednesday 14:00 – 16:00

Viruses 4

CONTRIBUTED PAPER Wednesday 14:00 **114**

Population structure of *Spodoptera litura* multicapsid nucleopolyhedroviruses from Pakistan

Ghulam Ali^{1,2}, *Marleen Henkens*², *Elio Schijlen*³, *Wopke van der Werf*⁴, *Just M. Vlak*²

¹CABI Rawalpindi, Pakistan; ²Laboratory of Virology and ⁴Centre for Crop Systems Analysis, Wageningen University, Wageningen, The Netherlands; ³Bioscience, Wageningen UR, Wageningen, The Netherlands

Correspondence: just.vlak@wur.nl

The cotton leaf worm, *Spodoptera litura*, is an emerging pest in cotton, rice and vegetable crops in Pakistan. Twenty-two nucleopolyhedroviruses were isolated from this insect from two

provinces in Pakistan. DNA restriction analysis indicated that all isolates were closely related to the G2 strain of SpltNPV from China (Pang *et al.*, 2001) and distantly related to a reference isolate, SpltNPV-G1, obtained from the baculovirus depository in Darmstadt, Germany. The latter isolate is more closely related to *S. littoralis* MNPV (SpliNPV) (Breitenbach *et al.*, 2013) confirming the existence of at least two clades within the Splt/SpliNPV complex. Comparison of restriction enzyme profiles of the 22 SpltNPV-PAK isolates indicated the existence of at least three genogroups within the SpltNPV-PAK isolates. These three genogroups did not coincide with the regional distribution of the isolates suggesting considerable virus drift. Complete DNA sequencing of SpltNPV-G1 and four SpltNPV-PAK isolates (BNG, Tax1, SFD1 and HRP6) confirmed the close genetic relationship among these isolates, as well as among SpltNPV-G1 and SpliNPV. Bioassays demonstrated no significant differences in median survival time (ST₅₀) among 3rd instar larvae exposed to different SpltNPV isolates from Pakistan (SpltNPV-PAK). The lethal doses (LD₅₀) of the (randomly) chosen isolate SpltNPV-PAK-BNG and the reference isolate SpltNPV-G1 in 3rd instar *S. litura* larvae were not significantly different. However, the median survival time (ST₅₀) was significantly lower for SpltNPV-PAK-BNG compared to SpltNPV-G1. Infected larvae consumed less food than uninfected larvae. These results indicate that SpltNPV-PAK-BNG offers potential for biological control of *S. litura* in Pakistan.

CONTRIBUTED PAPER Wednesday 14:15 **115**

Genome sequence and environmental tolerance of a granulovirus mutant that produces abnormally large occlusion bodies

*Madoka Nakai*¹, *Robert L. Harrison*², *Haruaki Uchida*¹, *Yasuhiro Kunimi*¹

¹Tokyo University of Agriculture and Technology, Saiwai, Fuchu, Tokyo 183-8509, Japan; ²Invasive Insect Biocontrol and Behavior Laboratory, USDA Agricultural Research Service (USDA-ARS), Beltsville Agricultural Research Center, 10300

Baltimore Avenue, Beltsville, MD 20705, USA

Correspondence: madoka@cc.tuat.ac.jp

Occlusion bodies (OBs), a diagnostic feature of family *Baculoviridae*, are comprised of a viral matrix protein that occludes virions in a paracrystalline array. OBs protect virions from environmental degradation and are the basis for baculovirus biopesticides. We isolated a novel *Betabaculovirus* isolate, *Adoxophyes orana* granulovirus isolate M (AdorGV-M) from Miyazaki, Japan, that produces abnormal OBs. AdorGV-M OBs are cuboidal in shape and reach a maximum diameter of 2.0 μm , whereas the reference isolate of AdorGV from UK (AdorGV-E) produces ellipsoid, 0.5 μm -long OBs typically observed for granuloviruses. AdorGV-M OBs were also found to be more resistant to inactivation by UV light and heat and to persist longer in the field than AdorGV-E OBs. To identify specific gene sequences that correlate with the abnormal OB morphology and greater environmental stability of AdorGV-M, the full genome sequence of AdorGV-M (99,507 bp) was compared to AdorGV-E and several AdorGV isolates from other regions in Japan. AdorGV-M exhibited 99.7% sequence identity with AdorGV-E, and 66 of 121 shared putative ORFs exhibited 100% predicted amino acid sequence identity between the two AdorGV genomes. No difference was observed in the *granulin* matrix protein gene, but other candidate OB morphology genes, including *pep-p10*, differed in sequence between AdorGV-M and other AdorGV isolates. These genes constitute a starting point in further studies on the molecular basis of the properties of AdorGV-M OBs.

CONTRIBUTED PAPER Wednesday 14:30 **116****The complete genome of *Aedes sollicitans* nucleopolyhedrovirus *Omaththage P. Perera*¹, James J. Becnel²**¹Southern Insect Management Research Unit, USDA-ARS, Stoneville, MS 38776; ²Mosquito and Fly Research Unit, CMAVE, USDA-ARS, Gainesville, FL 32606Correspondence: op.perera@ars.usda.gov

The complete genome of *Aedes sollicitans* Deltabaculovirus (AesoNPV) was assembled using short sequence reads obtained with Illumina HiSeq2000 platform. The genome was 84,974 bp and contained 75 open reading frames (ORFs), of which 45 ORFs were highly similar to *Culex nigripalpis* NPV (*CuniNPV*). The remaining 30 ORFs did not have any similarity to sequences available in the public databases. Genes conserved between *AesoNPV* and *CuniNPV* included all six *per os* infectivity genes, *vp1054*, all late expression factor except *lef-5*, the occlusion body protein, and most envelope proteins. Lack of a DNA polymerase gene (similar to *AcMNPV* ORF65 and *CuniNPV* ORF 91) was a notable exception in the *AesoNPV* genome. Compared to *CuniNPV* genome, *AesoNPV* genome is smaller in size and has substantial differences in gene organization.

CONTRIBUTED PAPER Wednesday 14:45 **117-STU****Lake Sinai Virus (LSV) diversity in Hymenoptera***Diane Bigot*¹, *Elisabeth A. Herniou*¹, *Nicolas Galtier*², *Philippe Gayral*¹¹Institut de Recherche sur la Biologie de l'Insecte, UMR 7261, CNRS, Université François-Rabelais, 37200 Tours, France; ²Institut des Sciences de l'Evolution de Montpellier, UMR 5554, CNRS, Université Montpellier 2, Place E. Bataillon, 34095 Montpellier, FranceCorrespondence: diane.bigot@etu.univ-tours.fr

Non-model animal species are a potential source of unknown viruses. This is especially important in the context of viral emergence. To describe the evolutionary origins of new RNA insect viruses, our study used Next Generation Sequencing technologies (NGS) for virus discovery. Thirty harvester ants (six species from the *Messor* genus) and thirteen wild bees (three species of the *Halictus* genus) were sampled in the fields and individual transcriptomes (mRNAs) were sequenced by Illumina technology to produce about 580 million reads. To obtain a list of viral contigs from raw transcriptomes, an innovative bioinformatics pipeline was developed. First, transcriptome assemblies were followed by a step of efficient protein homology detection with HMM profiles against available public protein databases. Next, viral taxonomy affiliation was associated with the predicted proteins. Then, new viral genomes were determined using a combination of contig re-assemblies and reads mapping on the new viral sequences. Finally, phylogenetic analyses were performed to confirm the taxonomic affiliations of these new viruses. Seven complete viral genomes close to Lake Sinai Virus (LSV) were reconstructed for three harvester ants and three wild bees. Phylogenetic analyses showed these new viruses shared a common ancestor with five previously described LSVs reported only in the honeybees *Apis mellifera*. Honeybees and wild bees are among the most economically important insects as they provide pollination service for food production. This discovery opens new perspectives on virus transmission routes between domestic bees, wild bees and ants.

CONTRIBUTED PAPER Wednesday 15:00 **118****Genomic landscape of AcMNPV adaptation***Aurélien Chateigner*, *Cindy Pontleve*, *Carole Labrousse*, *Elisabeth A**Herniou*

Institut de Recherche sur la Biologie de l'Insecte, UMR CNRS 7261, Université François Rabelais de Tours, Faculté des Sciences et Techniques, Avenue Monge - Parc Grandmont, 37200 Tours, France

Correspondence: elisabeth.herniou@univ-tours.fr

Genetic variation underpins the evolutionary process of adaptation. As populations become adapted to different environments, they diverge from one another. Baculoviruses infecting different host species thus usually belong to different species. Yet some viruses, such as *Autographa californica multiple Nucleopolyhedrovirus* (AcMNPV), have retained the capacity of infecting many host species. To understand how AcMNPV could draw on standing genetic variation to adapt to different host species we undertook an experimental evolution protocol. A highly polymorphic AcMNPV population was passaged 10 times through 4 different host species of various susceptibilities. We then characterised the genetic make up of the original and evolved baculovirus populations by ultra-deep Illumina sequencing. Using a population genomics approach, we then estimated the genetic diversity of each of the evolved populations and their divergence from one another. We found that the lines that evolved on the same host species were more similar to one another than to other lines, showing that the experimental evolution did lead to specific adaptation. Furthermore, we found a general diminution of genetic diversity compared to the ancestral population. However, none of the evolved populations became clonal. Lastly the viral lines that could adapt to more resistant host retained higher genomic diversity than those that did not. This suggests that indeed standing genetic variation is an important component of baculovirus adaptation.

CONTRIBUTED PAPER Wednesday 15:15 **119****In search of the ancestor of banchine polydnaviruses***Catherine Béliveau*¹, *Alejandro Cohen*², *Don Stewart*¹, *Georges Periquet*³, *Abdelmadjid Djoumad*⁴, *Lisa Kuhn*⁴, *Don Stoltz*⁵, *Brian Boyle*⁵, *Anne-Nathalie Volkoff*⁶, *Elisabeth A. Herniou*³, *Jean-Michel Drezen*³, *Michel Cusson*¹¹Natural Resources Canada, Canadian Forest Service, Laurentian Forestry Centre, Quebec City, Quebec, Canada; ²Proteomics Core Facility, Dalhousie University, Halifax, Nova Scotia, Canada; ³Institut de Recherche sur la Biologie de l'Insecte (IRBI), Université François-Rabelais et CNRS UMR 7261, Tours, France; ⁴Department of Microbiology and Immunology, Dalhousie University, Halifax, Nova Scotia, Canada; ⁵Institut de Biologie Intégrative et des Systèmes, Université Laval, Quebec City, Quebec, Canada; ⁶UMR 1333 INRA, Université Montpellier, "Diversité, Génomes, Interactions Microorganismes-Insectes" (DGIMI), Montpellier, FranceCorrespondence: michel.cusson@nrcan.gc.ca

Polydnaviruses form a group of unconventional dsDNA viruses transmitted by endoparasitic wasps during egg laying into caterpillar hosts, where viral gene expression is essential to immature wasp survival. A copy of the viral genome is present in wasp chromosomes, thus ensuring vertical transmission. Recent work indicates that the two recognized polydnavirus taxa, bracovirus and ichnovirus, are derived from distinct viruses whose genomes integrated into the genomes of ancestral wasps. However, the identity of the ichnovirus ancestor is unknown and questions remain regarding the possibility that the two described ichnovirus subgroups, banchine and campoplegine ichnoviruses, have distinct origins. To address the latter question, we used genomic, proteomic and transcriptomic analyses to characterize particle proteins of the banchine *Glypta fumiferanae* ichnovirus

and the genes encoding them. Our study provides unequivocal evidence that the two ichnovirus types are derived from related viral progenitors. This suggests that morphological and genomic differences observed between them, including features unique to banchine ichnovirus genome segments, result from evolutionary divergence either before or after their endogenization. Strikingly, analysis of selected wasp genomic regions revealed genes presumed to be part of the replicative machinery of the progenitor virus, shedding new light on the likely identity of this virus. Homology searches pointed to nucleocytoplasmic large DNA viruses as the likely source of these genes, which could well play a role in ichnovirus replication as they were overexpressed in the virogenic tissue.

CONTRIBUTED PAPER Wednesday 15:30 **120**

Construction and rescue of a synthetic baculovirus, AcMNPV-WIV-Syn1.0, that retains the properties of the parental virus

Yu Shang, Fei Deng, Gengfu Xiao, Dianhai Hou, Kai Pan, Manli

Wang, Hualin Wang, Zhihong Hu

State Key laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, P.R. China

Correspondence: huzh@wh.iov.cn

Baculoviruses contain a large circular DNA genome ranging from 80-180 kb and have been widely used as bio-control agents and protein expression vectors. To date, recombinant baculoviruses have been constructed based on homologous recombination in either insect cells or in *E. coli*. Here we report the first synthesis of AcMNPV, the prototype baculovirus, via transformation-associated recombination (TAR) in *Saccharomyces cerevisiae*. First, 45 overlapping fragments each of ~3 kb covering the entire genome of AcMNPV C6 were amplified by PCR. Then three rounds of TAR were performed step by step in *S. cerevisiae* to assemble the fragments into nine constructs each containing ~15 kb of the viral genome, then into three constructs each containing ~45 kb viral DNA and finally into one having the entire AcMNPV genome. The final construct with the correct sequence was used to transfect Sf9 cells and the recovered virus was designated AcMNPV-WIV-syn1.0. The synthetic genome is 145,258 bp in size containing the complete genome sequences of AcMNPV except *hr4a*, which was replaced by an 11.5 kb cassette of bacterial and yeast artificial chromosomal elements. The results of one-step growth curve, electron microscopy and oral infectivity showed that AcMNPV-WIV-syn1.0 had similar biological properties to the parental virus. The study proved the concept that a baculovirus can be synthesized *de novo* from parental sequences. In comparison to traditional recombination, the new method allows manipulating the baculoviral genome at multiple loci at one time and may likely be extended to other large DNA viruses such as poxviruses and hepersviruses. Currently, we are deleting/replacing multiple genes from the genome, which can be further used to study host range, identifying a baculovirus mini-genome, as well as improving it as an expression vector.

CONTRIBUTED PAPER Wednesday 15:45 **121**

Generating a host ranges extended recombinant baculovirus

Tzongyuan Wu, Chao-Yi Teng, Mean-Shine Chen

Department of Bioscience Technology, Chung Yuan Christian University, Chung Li, Taiwan

Correspondence: tyw@cycu.edu.tw

Baculovirus expression system (BEVS) has been one of the versatile platforms for the production of recombinant proteins. Combination of multiple promoters or IRES elements were improved methods for expressing multiple genes from a single

baculovirus vector, and made it possible to express multiple proteins simultaneously in a single recombinant virus. However, most baculoviruses have restricted, narrower host ranges, which is one of the important limitations for its applications in using insect larvae a bioreactor. We previously co-infected the AcMNPV and MaviMNPV into Sf21 cells and isolated the hybrid virus (Ac-Mv-bac), which could infect both host cells of AcMNPV and MaviMNPV. To further extend the host range, we have successfully isolated the "ABM" virus, which can replicate in Sf21, Mv532 and BmN cells, by using the same approach. Here, we utilized the ABM-Bac baculovirus expression system to express H5N1 avian flu viruslike particle (VLP) comprises HA, NA, and M1 proteins in the three hosts. Besides, we also provided an alternative approach to produce multiple proteins from the separated locus within the viral DNA. We had constructed six transfer vectors with different recombination locus and various fluorescence markers to establish a multiple locus-based polycistronic baculovirus expression vector, named as poly-Bac. In order to optimize the production of secreted or membrane bounded proteins, incorporation of the translation initiation factor- eIF4E and molecular chaperones Calreticulin and β -synuclien into poly-Bac system were also established.

NEMATODE WORKSHOP Wednesday 14:00 – 15:00

Nematodes Workshop

WORKSHOP PAPER Wednesday 14:00 **122**

Entomopathogenic nematode species in insect pest suppression: Can their use be optimized by proper application approaches?

Harry K. Kaya¹, Selcuk Hazir², David Shapiro-Ilan³

¹Department of Entomology and Nematology, University of California, Davis, CA 95616, USA; ²Department of Biology, Faculty of Arts and Sciences, Adnan Menderes University, Aydin, TURKEY; ³USDA-ARS, SE Fruit and Tree Nut Research Laboratory, Byron, GA 31008, USA

Correspondence: hkkaya@ucdavis.edu

Successful application of entomopathogenic nematodes (EPNs) for insect suppression requires an understanding of nematode behavior, insect biology, and environmental conditions. One of the basic principles in EPN application is matching the right species with the target insect. Suitability to a target pest depends on the nematode's innate virulence as well as other factors (such as foraging strategy). The concept of mixing two EPN species or the application of EPNs with a stressor such as a low concentration of an insecticide has enhanced insect control. Optimization of environmental conditions and proper timing of EPN applications (such as avoiding harmful UV radiation or desiccation) is critical for biocontrol success. Besides these basic procedures, genetic improvement of EPNs to desiccation or heat tolerance, the addition of adjuvants, and improved nematode formulations can enhance insect control. EPN persistence and efficacy can be improved by the use of insect cadavers containing EPNs. More recently, the use of live insects infected with EPNs was found to enhance insect control. Before dying, the live infected insects move to an area where the target pest resides. Then the EPNs in the dead insects produce infective juveniles that infect target insects in the vicinity. This concept has been referred to as "living insect bombs" and is particularly useful against insect pest species that occur in cryptic habitats. Finally, we have found that insect cadavers with nematodes are protected from being consumed by insect scavengers and this protection enhances nematode reproduction.

WORKSHOP PAPER Wednesday 14:15 **123****EPNs from lab to field against insect pests of fine turfgrass:
Overcoming obstacles in research and implementation**Albrecht M. Köppenhöfer¹, Olga S. Kostromytska¹, Shaohui Wu¹,
Benjamin A. McGraw², Lemma Ebssa³¹Department of Entomology, Rutgers University, New Brunswick, NJ 08901, USA; ²Department of Plant Science, Pennsylvania State University, University Park, PA 16802, USA; ³Institute for Health, Health Care Policy, and Aging Research, Rutgers University, New Brunswick, NJ 08901, USA

Implementation of entomopathogenic nematode (EPN) use against insect pests on golf courses in the USA is challenging due to high turf quality standards. We will discuss how to streamline the development of meaningful data through lab and greenhouse to the field and how to optimize efficacy to make EPN a viable management option. Black cutworm, *Agrotis ipsilon* (Lepidoptera: Noctuidae), larvae damage golf course tees and putting greens. Because they can easily be reared we conducted extensive laboratory screening. In 30-ml plastic *Heterorhabditis megidis* was the most virulent species. But in 500-ml cups with bentgrass growing on soil, *S. carpocapsae* tended to be most effective species. Field experiments, in 0.1 m²-enclosures to prevent BCW escape and bird predation, confirmed *S. carpocapsae* as the most effective species due to a combination of control rates, speed of control, and consistency. Twice daily light irrigation and split applications further improved EPN efficacy to excellent levels. Nonetheless, EPN will likely continue to be limited wherever cheaper and easier-to-use highly effective synthetic insecticides can be used. Annual bluegrass weevil (ABW), *Listronotus maculicollis*, larvae damage all short mown areas on golf courses. Screening for effective EPN for this pest was challenging since it cannot be easily reared. Ultimately, *S. carpocapsae* was the most promising EPN species but excellent field efficacy could only be achieved through combination with imidacloprid (used at the same time for white grub control) and split applications. Widespread issues with broad insecticide resistance will likely make EPN a viable options for ABW management.

WORKSHOP PAPER Wednesday 14:30 **124****Plant scream, insect succumb: From concepts to applications***I. Hiltbold*

Hawkesbury Institute for the Environment, University of Western Sydney, Penrith, NSW, Australia

Insect herbivory induces synthesis and release of specific volatile compounds in plants. These volatiles have been shown to be highly attractive to natural enemies of the herbivores, such as predators, parasitic wasps or entomopathogenic nematodes. In maize, the volatiles emitted upon feeding by leaf- or root-feeding arthropods have been particularly well studied. Among several, some key compounds mediating these so-called tritrophic interactions have been identified. Moreover, several genes and biochemical pathways responsible for the production of the emitted volatiles have been elucidated. These advances in understanding the volatile emission and its ecological signaling open novel ways to modify and/or exploit plant volatile blends in order to enhance their attractiveness to natural enemies. Beside this plant manipulation, different other approaches are explored in order to better control belowground herbivory exploiting belowground volatile signaling. For instance, entomopathogenic nematodes have been selected for a better responsiveness to belowground cues or techniques to lure the foraging insect

herbivore are currently developed. Most of these manipulations could be simultaneously used for the benefit of agriculture.

Wednesday 16:30 – 18:00

POSTERS**Bacteria**POSTER Wednesday 16:30 **BA-1-STU****Novel mosquitocidal activity of strains LBIT-980 and LBIT-1217 of
*Bacillus thuringiensis***Mariana Fernández-Navarro¹, Cristina Del Rincón-Castro², Jorge E.
Ibarra¹¹Department of Biotechnology and Biochemistry, CINVESTAV-Irapuato, Gto. Mexico; ²Life Sciences Division, University of Guanajuato, Irapuato, Gto. MexicoCorrespondence: jibarra@ira.cinvestav.mx

Bacillus thuringiensis ssp. *israelensis* and *Lysinibacillus sphaericus* are successfully used to control mosquito populations. However, the possibility of finding novel mosquitocidal activities is still interesting and feasible. In this work, two strains of *B. thuringiensis* were selected by preliminary bioassays, showing activity against *Aedes aegypti* larvae: LBIT-980 (serovar *kim*) isolated from soil; and LBIT-1217 (not serotyped), isolated from vermicompost. LBIT-980 showed a collapsed-balloon shaped crystals which contains the Cry59Aa1 protein; while LBIT-1217 strain showed a typical bipyramidal crystal morphology. Identification of Cry protein(s) is underway. Quantitative bioassays were performed following the standard procedure, using early fourth instar larvae of *A. aegypti*, to determine the toxicity levels. The LC₅₀ of spore-crystal complexes were 6.53 µg/mL and 13.42 µg/mL for LBIT-980 and LBIT-1217 strains, respectively; whereas using pure crystals LC₅₀ of 2.85 µg/ml and 3.99 µg/mL were estimated. Although these toxicity level are far lower than those shown by Bti, they are comparable to those shown by the Bt toxin components tested separately. None of both strains showed the presence of Cyt toxins, neither in the gene content nor in the protein content. That is why bioassays using mixtures of LBIT-980/Cyt and LBIT-1217/Cyt are underway. As part of the characterization, plasmids patterns from the LBIT-980 strain are similar to those of serovar *kim* type strain, and LBIT-1217 shows the presence of four plasmids, two larger than 16kb and two more with approximate weights of 10.5 kb and 14 kb.

POSTER Wednesday 16:30 **BA-2-STU****Inoculation and translocation of the spore-crystal complex from
Bacillus thuringiensis in vascular tissue of bean plants**Rosalina García-Suárez¹, Cristina Del Rincón-Castro², Jorge E.
Ibarra¹¹Department of Biotechnology and Biochemistry, CINVESTAV-Irapuato, Gto. Mexico; ²Life Sciences Division, University of Guanajuato, Irapuato, Gto. MexicoCorrespondence: jibarra@ira.cinvestav.mx

Studies in cotton, soybean, corn, sugar cane, cabbage, etc., have reported that *Bacillus thuringiensis* (Bt) has been successful in endophytic colonization. The efficient colonization of Bt in seedling roots suggests that this could be the main route to gain entry into the plant and, subsequently, migrate through the xylem

to aerial tissues. In this paper, Bt subsp. *kurstaki* HD-73 was transformed with the green fluorescent protein reporter gene in order to obtain fluorescent vegetative cells and spores. The spore-crystal complex was inoculated in bean plants via two mechanisms: in the rhizosphere of plants grown in conventional substrate and in a hydroponic salt solution. Fluorescent spores were observed in the sap of plants inoculated with the spore-crystal complex, which were absent in the sap of non-inoculated plants. Interestingly, mortality was observed when *Trichoplusia ni* neonate larvae were fed with leaves of Bt-inoculated plants. To determine the possible lethal effect of recombinant strain carrying the Cry1Ac toxin, dead larvae were homogenized and cultured, recovering the fluorescent strain. As above, larvae fed on non-inoculated plants showed absence of Bt. Moreover fluorescent spores were observed within the aerial tissue of inoculated plants. These results suggest that the spore-crystal complex inoculated in the rhizosphere of bean plants is able to penetrate and translocate through the vascular system to aerial parts of the plant, without losing its toxicity. The translocation mechanism is still unknown but its study and improvement may innovate the way Bt is used in the field.

POSTER Wednesday 16:30 **BA-3-STU**

Diversity of bacteria composition of spined soldier bug, *Podisus maculiventris* (Hemiptera: Pentatomidae) digestive system
E. M. Romagnoli¹, C. A. Dunlap², L. B. Flor-Weiler², T. A. Coudron³, A. P. Rooney²

¹“Luiz de Queiroz” College of Agriculture, University of Sao Paulo, Piracicaba, Sao Paulo, 13418900, Brazil; ²National Center for Agricultural Utilization Research, Agricultural Research Service, U.S. Department of Agricultural, Peoria, IL, 61604, USA; ³Biological Control of Insects Research Service, U.S. Department of Agricultural, Columbia, MO, 65203

Correspondence: emilianaromagnoli@gmail.com

Spined soldier bug is an important biological control agent that preys on eggs and larvae of important forest and agricultural pests. Attempts at mass production of the insect have not been successful due to limited success of artificial diets used for long-term colony rearing. One possible solution is to incorporate probiotic microorganisms into artificial diets. Unfortunately, virtually nothing is known concerning which microorganisms are effective probiotic agents in insects. As a first step in answering this question for the spined soldier bug, we analyzed the gut microbial community of wild and laboratory populations using next-generation sequencing of 16S ribosomal RNA (rRNA) gene amplicons. Bacteria found in both populations were dominated by Proteobacteria, followed by Firmicutes, Bacteroidetes, and Actinobacteria although differences were apparent. Laboratory populations had a higher composition of Proteobacteria (96%) than those collected from the wild (74%). On the other hand, wild populations showed higher levels of Firmicutes (8% vs 3%) as well as Bacteroidetes (6% vs 0.2%), as well as the proportion of unclassified species (9% vs 1%). Interestingly, the community composition differences concerning the minor community components (i.e., Firmicutes, Bacteroidetes and unclassified bacteria) could represent potential probiotic species on the basis of comparison to studies in other organisms. The implications of these findings for designing an improved artificial diet are discussed.

POSTER Wednesday 16:30 **BA-4-STU**

Use of NGS to assess the effect of antibiotics on the bacterial community of insects

Diana Wilches^{1,2}, Kevin D. Floate², Paul Coghlin²

¹University of Lethbridge; ²Agriculture and Agri-Food Canada, Research Centre, Lethbridge, AB

Correspondence: Diana.Wilches@agr.gc.ca

Symbiotic bacteria are common in insects and can influence the biology, reproduction and survival of their host. *Wolbachia* are among the most common of these symbionts that are often studied by applying antibiotic treatments to the host to eliminate the infection and, assessing the effects on host fitness. Antibiotic treatments may also affect other bacteria associated with the host, but this issue is rarely assessed. We used next-generation sequencing (NGS) methods to characterize the effect of antibiotic treatments on *Wolbachia* and non-*Wolbachia* bacteria associated with *Drosophila suzukii*. Efforts to distinguish the effects of *Wolbachia* and non-*Wolbachia* bacteria on host biology may lead to improved methods of insect pest control.

POSTER Wednesday 16:30 **BA-5**

Status of resistance to Bt cotton in China: Cotton bollworm and pink bollworm

Yulin Gao¹, Chenxi Liu¹, Yidong Wu², Kongming Wu¹

¹State Key Laboratory for Biology of Plant Disease and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, PR China; ²Department of Entomology, College of Plant Protection, Nanjing Agricultural University, Nanjing 210095, PR China

Correspondence: gaoyulin@caas.cn

Transgenic cotton that expresses a gene derived from the bacterium *Bacillus thuringiensis* (Bt) has been deployed for combating *Helicoverpa armigera* and *Pectinophora gossypiella* since 1997 in China. The pest management tactics associated with Bt cotton have resulted in a drastic reduction in insecticide use. However, evolution of resistance by the pests threatens the continued success of Bt cotton. The development of resistance to Bt is of great concern, and there is a vast body of research in this area aimed at ensuring the continued success of Bt cotton. Here, we review studies on the evolution of Bt resistance in these two bollworms, focusing on commercial release of Bt cotton varieties in China, the biochemical and molecular basis of Bt resistance. We also discuss resistance management strategies, and monitoring programs implemented in China and other countries.

POSTER Wednesday 16:30 **BA-6**

Evidence of field-evolved resistance of *Spodoptera frugiperda* to Bt corn expressing Cry1F in Brazil that is still sensitive to modified Bt toxin

Lima A. Anabele^{1,5}, Monnerat Rose¹, Martins Erica², Macedo Cristina^{1,3}, Queiroz Paulo², Praça Lilian¹, Soares C. Marcelo², Moreira Helio¹, Grisi Isabella¹, Silva Joseane¹, Soberon Mario⁴, Bravo Alejandra⁴

¹Embrapa Recursos Genéticos e Biotecnologia, Brasília, Distrito Federal, Brazil; ²Instituto Mato-Grossense do Algodão, Cuiabá, Mato Grosso, Brazil; ³Departamento de Microbiologia, Universidade de Brasília, Brasília, Distrito Federal, Brazil; ⁴Instituto de Biotecnologia, Universidad Nacional Autónoma de México, Cuernavaca, Morelos, Mexico; ⁵University Center of Brasília, Distrito Federal, Brazil

Correspondence: anabelebio@gmail.com,
anabele.lima@uniceub.br

Brazil ranked second only to the United States in hectares planted to genetically modified crops in 2013. Recently corn producers in the Cerrado region reported that the control of *Spodoptera*

frugiperda with Bt corn expressing Cry1Fa has decreased, forcing them to use chemicals to reduce the damage caused by this insect pest. A colony of *S. frugiperda* was established from individuals collected in 2013 from Cry1Fa corn plants (SfBt) in Brazil and shown to have at least more than ten fold higher resistance levels compared with a susceptible colony (Sflab). Laboratory assays on corn leaves showed that in contrast to Sflab population, the SfBt larvae were able to survive by feeding on Cry1Fa corn leaves. The SfBt population was maintained without selection for eight generations and shown to maintain high levels of resistance to Cry1Fa toxin. SfBt showed higher cross-resistance to Cry1Aa than to Cry1Ab or Cry1Ac toxins. As previously reported, Cry1A toxins competed the binding of Cry1Fa to brush border membrane vesicles (BBMV) from Sflab insects, explaining cross-resistance to Cry1A toxins. In contrast Cry2A toxins did not compete Cry1Fa binding to Sflab-BBMV and no cross-resistance to Cry2A was observed, although Cry2A toxins show low toxicity to *S. frugiperda*. Bioassays with Cry1AbMod and Cry1AcMod show that they are highly active against both the Sflab and the SfBt populations. The bioassay data reported here show that insects collected from Cry1Fa corn in the Cerrado region were resistant to Cry1Fa suggesting that resistance contributed to field failures of Cry1Fa corn to control *S. frugiperda*.

POSTER Wednesday 16:30 **BA-7**

Behavioural feeding responses of susceptible and resistant *Trichoplusia ni* larvae to *Bacillus thuringiensis*

Alida F. Janmaat, Mandy Gelderman, Martin Muermann
Biology Department, University of the Fraser Valley, Abbotsford,
BC, Canada

Correspondence: alida.janmaat@ufv.ca

Exposure of *Trichoplusia ni* larvae to sublethal levels of Bt have differential effects on *Bacillus thuringiensis* resistant and susceptible *T. ni*. Tolerance to Bt is induced in susceptible larvae and resistant larvae exhibit increased growth in response to sublethal Bt doses. These effects may modify the feeding behaviour of *T. ni* larvae and alter subsequent Bt exposure. The effects of pre-exposure to sublethal Bt concentrations on larval feeding behaviour of resistant and susceptible *Trichoplusia ni* larvae were examined through diet choice tests. Both control and pre-exposed susceptible larvae avoided diet treated with Bt, despite the reduction in mortality experienced by the pre-exposed larvae. Resistant larvae experienced an increase in weight gain when exposed to Bt, and exhibited an increase in avoidance of diet with Bt when given a choice of diets. Feeding observations suggest that both susceptible and resistant *T. ni* larvae modify the impact of Bt exposure through behavioural avoidance and compensatory feeding.

POSTER Wednesday 16:30 **BA-8**

Discovering novel Bt toxins for better bio-pesticides and biotech crops

Jim X. J. Fang^{1,2}, *W. F. Zhang*³, *Y. Zhou*², *P. T. Gong*²
¹The HITAR Institute Canada Inc., Richmond, British Columbia,
Canada; ²Hainan Institute of Tropical Agricultural Resources,
Sanya, Hainan, China; ³College of Life Sciences, Hainan Normal
University, Haikou, Hainan, China

Correspondence: jim.x.j.fang@hitar.org

Bacillus thuringiensis, *B.t.*, is one of the most famous bacterium that generates many virulence factors including phospholipases, hemolysins, enterotoxins, exotoxins and endotoxins. Since discovered in 1901, humans have always benefited from this soil saprophyte. The project titled China National Bt Collection

Initiative (BtSRI) was initiated by HITAR in 2005, which aimed to sample the soils across mainland of China for isolating the *Bacillus* and identifying the strains of *Bacillus thuringiensis*. So far, 27,250 *Bacillus* isolates were collected in stock at HITAR and 2137 isolates were identified as *B.t.* strains. More than 150 strains were characterized in crystal shape, SDS-PAGE, plasmid profiles and cry genotypes, Bioassay etc., of which 30 strains being working strains in HITAR are applying to whole genome sequencing to characterize the genome and discover novel *B.t.* toxins. Five strains, S2160-1, S2480-1, S2096-2, S3299-1, W015-1 and 2012-2, were proposed to be new model strains. *B.t.* strains, W015-1, S1478-1 and S3299-1 having new high toxic to *Lepidopteran*, were proposed to be alternative to *Btk*, while S2160-1, a new high mosquitocidal isolate being alternative to *Bti*. S2096-2 being significantly highly toxic to the hookworm (*Necator americanus*) might be applied to produce biocides. S3012-2 has significant toxicity against corn rootworm. The Genomes of the selected 36 isolates including above mentioned S2160-1, S2096-2, S2480-1, S3299-1 and S3012-2 were sequenced to characterize the strains and to discover new toxins, which show the potentials for commercial application both in engineering bacteria and genetically modified crops.

POSTER Wednesday 16:30 **BA-9**

Toxicity and interaction of Cry1 proteins from *Bacillus thuringiensis* in *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae)

*Manoel Victor F. Lemos*¹, *Isis Sebastião*¹, *Camila S Figueiredo*¹, *Ana Rita N Lemes*¹, *Ricardo A Polanczyk*², *Janete A Desidério*¹
Faculdade de Ciências Agrárias e Veterinárias, UNESP – Univ.
Estadual Paulista Júlio de Mesquita Filho; ¹Departamento de
Biologia Aplicada à Agropecuária; ²Departamento de
Fitossanidade. Rod. Prof. Paulo Donato Castellane, km. 5,
CEP14884-900, Jaboticabal, São Paulo, Brasil
Correspondence: mvector@fcav.unesp.br

The cotton bollworm *Helicoverpa armigera* is the major pest in countries like that India, China and Australia. It causes many damages in several crops of the world. Reports about its introduction in Brazil showed this pest affected soybean, cotton, tomato and bean crops. *Bacillus thuringiensis* (Bt) toxins can be used to control this pest through its biopesticides proteins or under transgenic Bt crops protection. Reports about Bt use to control *H. armigera* are scarce in Brazil and the aim of this research was evaluate the toxicity and interaction of the *B. thuringiensis* proteins, Cry1Aa, Cry1Ab, Cry1Ac and Cry1Ca. To estimate the LC50 there were performed bioassays with *H. armigera* neonate larvae. The interaction was evaluated *in vitro* using competitive binding assays with the activated and biotinylated proteins and "brush border membrane vesicles" BBMV from the midgut larvae. The toxicity resulted in Cry1Ac protein was the most toxic followed by Cry1Ab and Cry1Aa proteins. The Cry1Ca showed no toxicity. Heterologous competitive binding assays revealed that Cry1Aa, Cry1Ab, Cry1Ac compete for a common receptor from the midgut larvae.

POSTER Wednesday 16:30 **BA-10**

Interaction of Vip3Aa42 and Cry1Ia10 proteins from *Bacillus thuringiensis* and toxicity to *Anticarsia gemmatilis* (Hübner, 1818) (Lepidoptera: Erebidae)

Suzana C. Marucci, *Janete A. Desidério*, *Manoel Victor F. Lemos*
Departamento de Biologia Aplicada à Agropecuária, Univ. Estadual
Paulista Júlio de Mesquita Filho, UNESP/Campus de Jaboticabal,
SP, CEP 14884-900, Brasil
Correspondence: mvector@fcav.unesp.br

Anticarsia gemmatalis (Hübner, 1818) (Lepidoptera: Noctuidae) is a soybean pest and is considered a risk to the Brazilian crops productivity. The insect in its larval instars, feed on the young leaves causing extensive damage to crops ranging from defoliation to the complete plant destruction. For the control of this pest, the use of Vip₃Aa and Cry₁Ia proteins become an excellent alternative, since they can be used on pest resistance management strategies. Therefore, this study aimed to the cloning and expression of Vip₃Aa₄₂ and Cry₁Ia₁₀ proteins from *Bacillus thuringiensis* (Bt) in *Escherichia coli*, in order to analyze the correlation between the binding to receptors through competition assays between the different toxins and the toxicity to *A. gemmatalis* larvae. Therefore, vip₃Aa₄₂ and cry₁Ia₁₀ genes were cloned into the pET SUMO vector, expressed in *E. coli* and the proteins toxicity were tested based on bioassays with neonate *A. gemmatalis* larvae. The BBMVs were prepared from the larvae mesenteron and competition assays were performed. The Vip₃Aa₄₂ and Cry₁Ia₁₀ proteins showed LC₅₀ of 239.2 ng/cm² and 246.2 ng/cm², respectively. Binding assays to BBMVs demonstrated that Vip₃Aa₄₂ and Cry₁Ia₁₀ proteins binding effectively to receptors present in the larvae midgut and, therefore, there was a correlation between the toxicity and the binding to receptors for the population of *A. gemmatalis*. Thus, the combination of Vip₃Aa₄₂ and Cry₁Ia₁₀ proteins is indicated for the production of biological insecticidal, as well as for the transgenic plants construction to overcome the resistance emergence by *A. gemmatalis* population to Bt toxins

POSTER Wednesday 16:30 **BA-11-STU**

Parallels between the Cry41Aa parasporin and insecticidal Bt toxins

Barbara Domanska, Michelle West, Neil Crickmore

Department of Biochemistry, School of Life Sciences, University of Sussex, Falmer, Brighton, BN1 9QG

Correspondence: bd73@sussex.ac.uk

In this study the cytotoxic activity associated with the Cry41Aa human cancer cell-active toxin of *Bacillus thuringiensis* (Bt), also known as Parasporin-3, was characterized. Our initial data were consistent with Cry41Aa being a pore-forming toxin in that rapid membrane damage was observed in susceptible cells and microscopic observation revealed cellular and nuclear swelling. The activation of apoptosis effectors Caspase 3/7 was not observed, although phosphorylation of p38 MAP kinase was. Interestingly, the extracellular depletion of divalent cations by different chelators prevented toxicity. This loss of toxicity could be rescued by the addition of metal ions such as calcium or zinc but not others such as magnesium or iron. Divalent cations have previously been implicated in the mode of action of both insecticidal Bt toxins and other parasporins where mechanisms other than pore formation were proposed to lead to cell death. We will discuss models designed to explain our results. In these models the divalent cations have several potential functions including a direct role in toxin binding, maintaining the structural integrity of the cell membrane and as a component of a cell signalling pathway that could lead to either cell death or cell repair. Although these models are based on our Parasporin / human cell assays they may be applicable to other Bt Cry toxin systems.

POSTER Wednesday 16:30 **BA-12-STU**

Unusual pore formation by a new *Bacillus thuringiensis* toxin

Eva Fortea Verdejo¹, Vincent Lemieux^{1,2}, Léna Potvin¹, Timothy Hey³, Xiaoping Xu³, Samantha Griffin³, Vimbai Chikwana³, David McCaskill³, Ken Narva³, Vincent Vachon¹, Jean-Louis Schwartz^{1,4}

¹Département de physiologie moléculaire et intégrative and Groupe d'étude des protéines membranaires, Université de Montréal, Montréal, QC, Canada; ²Département de biologie, Université de Sherbrooke, Sherbrooke, QC, Canada; ³Dow Agrosciences LLC, Indianapolis (IN 46268), IN, USA.; ⁴Centre SÈVE de recherche en sciences du végétal, Université de Sherbrooke, Sherbrooke, QC, Canada

Correspondence: jean-louis.schwartz@umontreal.ca

Typical three-domain *Bacillus thuringiensis* (Bt) insecticidal and nematocidal proteins, and Bt parasporins permeabilise receptor-free planar lipid bilayers (PLBs) by forming pores at doses in the 1–50 µg/ml range. At lower doses, pore formation may only take place when PLBs are enriched with purified target organism receptors or midgut brush border material. Under symmetrical 150 mM KCl conditions, the conductance of the pores is comprised between 10 and 500 pS and the pores are mildly selective for cations. Activation of the purified and solubilized proteins obtained from Bt inclusion bodies by trypsin, target organism gut proteases or gut juice is a necessary step for PLB permeabilisation.

DS1 is a new Bt protein which displays insecticidal activity *in vivo*. Contrary to other Bt toxins tested so far in PLBs, DS1 forms pores in receptor-free bilayers without the need of protease or target insect gut juice pretreatment, and at doses that are 2 to 3 orders of magnitude lower than those required for other Bt toxins under similar conditions. Pore formation by DS1 is highly pH-dependent; the conductance of the pores ranges between 100 and 450 pS under symmetrical 150 mM KCl conditions; they are cation-selective and display a complex kinetic behaviour. The molecular determinants of the mode of action of this new pore-forming Bt toxin appear therefore to differ from those reported before for other Bt toxins, which may imply a radically different structural organisation of the protein.

POSTER Wednesday 16:30 **BA-13-STU**

Construction & characterization of novel *Bacillus thuringiensis* Cry1-type genes with improved insecticidal activities

Jong Hoon Kim¹, Jae Young Choi², Pang Ying¹, Ha Kyu Baik¹, Seok Hee Lee¹, Woo Jin Kim², Yeon Ho Je¹

¹Department of Agricultural Biotechnology, Seoul National University, Seoul, Republic of Korea; ²Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul, Republic of Korea

Correspondence: KJH1018@snu.ac.kr

Crystals of proteinaceous insecticidal proteins, Cry proteins, produced by *Bacillus thuringiensis* (Bt) have been generally used to control insect pests. In this study, through the 3D structure prediction and accompanying mutagenesis study for the Mod-Cry1Ac, 7 and 16 amino acid residues from domain I and II, respectively, responsible for its insecticidal activity against larvae of *Plutella xylostella*, *Spodoptera exigua* and *Ostrinia furnacalis* were identified. To construct novel cry genes with enhanced insecticidal activity, we randomly mutated these 24 amino acid sequences by *in vitro* multi site-directed mutagenesis, resulting in a total of 34 mutant cry genes. For further characterization, these mutant cry genes were expressed as a fusion protein with polyhedrin using baculovirus expression system. SDS-PAGE analysis of the recombinant polyhedra revealed that expressed Cry proteins were occluded into polyhedra and activated to yield 65 kDa by trypsin. When the insecticidal activities of these mutant Cry proteins against larvae of *P. xylostella*, *S. exigua*, and *O. furnacalis* were assayed, they showed higher or similar insecticidal activity compared to those of Cry1Ac and Cry1C. Especially, among these 34 mutant cry genes, Mutant-N16 showed the

highest insecticidal activity against *P. xylostella*, *S. exigua* and *Ostrinia furnacalis* larvae. Therefore, Mutant-N16 is estimated to have the potential for the efficacious bioagent.

POSTER Wednesday 16:30 **BA-14-STU**

Development of a Cry toxin activity-improving method based on the directed evolution that targets ABCC2

Kazuhiro Imamura¹, Natsuko Nakajima¹, Haruka Endo^{1,2}, Ryoichi Sato¹

¹Graduate School of Bio-Applications and Systems Engineering Tokyo University of Agriculture and Technology, Koganei, Tokyo, Japan; ²Japan Society for the Promotion of Science Research Fellowship for Young Scientists

Correspondence: ryoichi@cc.tuat.ac.jp

ABC transporter C2 (ABCC2) is a functional receptor of Cry1A in lepidopteran insects, and thought to play an important role in the mechanism of action of Cry toxins. Since the interaction to the receptors are necessary to exert insecticidal activity for Cry toxin, the improvement of binding affinity to ABCC2 is expected to lead the insecticidal activity enhancement. Directed evolution is a method that can screen proteins that display higher binding affinity to a target protein. This research aims to assess the possibility of enhancement of the Cry toxins insecticidal activity by applying directed evolution that targets ABCC2. Cry8Ca has low toxicity to *Bombyx mori* larvae (>73 µg/g diet), and low binding affinity to *B. mori* ABCC2 (*BmABCC2*). Domain II loop regions of Cry toxins have been indicated to bind to receptors and effect to the insecticidal activity. Hence, at first, random mutations were introduced in successive 4 amino acid residues on Cry8Ca loop 3, and these mutants were displayed on T7 phage surfaces. Subsequently, a basic selection method to screen mutants that exhibit higher binding affinity to *BmABCC2* from the phage library were attempted to construct. Native phages containing few Cry1Aa-expressing phages which should bind to *BmABCC2* were subjected to the selection. After the selection, the percentage of Cry1Aa-expressing phages has increased 6-fold. This suggested that phages which possess mutant Cry toxins with improved binding ability to *BmABCC2* can be obtained through repeat of this selection. Selection of the phage library with Cry8Ca mutant has been started.

POSTER Wednesday 16:30 **BA-15**

Carbohydrate binding domain of Vip3Aa is involved in receptor binding

Kun Jiang¹, Yu Yuan¹, Dong-hui Hua¹, Tingting Wang, Yue-hua Chen^{1,2,3}, Jun Cai^{1,2,3}

¹Department of Microbiology, College of Life Sciences, Nankai University, Tianjin, China; ²Key Laboratory of Molecular Microbiology and Technology, Ministry of Education, Tianjin, China; ³Tianjin Key Laboratory of Microbial Functional Genomics, Tianjin, China

Correspondence: caijun@nankai.edu.cn

Vip3A, a soluble insecticidal protein produced during vegetative growth phase by *Bacillus thuringiensis*, possesses insecticidal activity against a wide spectrum of lepidopteran insects. In contrast to the situation with δ -endotoxins, little is known about insecticidal action mechanism of Vip3A. In our previous work, we found that a carbohydrate binding domain (CBD) exists on the C-termini 536 to 667 residues of Vip3Aa. In this study, in order to investigate the function of CBD, we create several truncated Vip3Aa proteins and Vip3Aa CBD site-directed mutants. Binding assays experiments showed that all the truncated proteins which contain the whole CBD, as well as CBD itself, still maintain binding

activity to sf9 cells and brush border membrane vesicles of *Spodoptera exigua*, while the truncated protein with incomplete CBD loses the binding activity. Moreover, bioassay results showed that the CBD site-directed mutants of Vip3Aa lost their toxicity. Furthermore, SDS-PAGE and western blotting analysis suggested that CBD mutant proteins were degraded in *E. coli*. These results demonstrate that CBD of Vip3Aa conceivably participates in toxin-receptor recognition and it is critical for maintaining the activity.

POSTER Wednesday 16:30 **BA-16**

Insecticidal spectrum and mode of action of the *Bacillus thuringiensis* Vip3Ca insecticidal protein

Joaquín Gomis-Cebolla¹, Iñigo Ruiz de Escudero^{2,3}, Maissa Chakroun¹, Natalia Mara Vera-Velasco¹, Patricia Hernández-Martínez¹, Carmen Sara Hernández-Rodríguez¹, Yolanda Bel¹, Baltasar Escriche¹, Primitivo Caballero^{2,3}, Juan Ferré¹

¹ERI de Biotecnología y Biomedicina (BIOTECMED), Universitat de València, Burjassot, Spain; ²Instituto de Agrobiotecnología, CSIC-UPNA, Gobierno de Navarra, Campus Arrosadía, Navarra, Spain;

³Laboratorio de Entomología Agrícola y Patología de Insectos, Universidad Pública de Navarra, Pamplona, Spain

Correspondence: juan.ferre@uv.es

The Vip3Ca protein, discovered in a screening of Spanish collections of *Bacillus thuringiensis*, was shown to be toxic to *Chrysodeixis chalcites*, *Mamestra brassicae* and *Trichoplusia ni* (Palma et al., 2012, Appl. Environ. Microbiol. 78:7163-5). In the present study, we have tested additional species and found three more lepidopteran species relatively susceptible to this protein: *Cydia pomonella*, *Grapholita molesta* and *Epehestia kuehniella*. Vip3Ca (a ca. 90 kDa protein) is processed to a ca. 60 kDa protein when incubated with midgut juice of larvae, no matter if they are susceptible (*M. brassicae*), moderately susceptible (*Agrotis ipsilon*) or non-susceptible (*Ostrinia nubilalis*) to this protein. This result suggests that the activation step is not critical in determining the susceptibility of an insect species. *In vivo* binding of Vip3Ca was performed by feeding *M. brassicae* larvae with Vip3Ca followed by detection with an anti-Vip3 protoxin polyclonal antibody. Histopathological inspection showed swelling of the epithelial cells with further disruption, which suggests that the mode of action of Vip3Ca is similar to that described for Vip3Aa. Comparative analysis of Vip3Ca binding to BBMV from tolerant and susceptible insects was performed.

POSTER Wednesday 16:30 **BA-17**

Binding to brush border vesicles and midgut processing of *Bacillus thuringiensis* Vip3Aa toxin to *Helicoverpa armigera* susceptible and Vip3Aa-resistant insects

Maissa Chakroun¹, Nuria Banyuls¹, Tom Walsh², Sharon Downes³, Rod Mahon³, Bill James², Juan Ferré¹

¹ERI of Biotechnology and Biomedicine (BIOTECMED), Universitat de València, Burjassot, Spain; ²Land and Water Flagship, CSIRO, Canberra ACT 2601; ³Agriculture Flagship, CSIRO, Narrabri NSW 2390

Correspondence: juan.ferre@uv.es

Two *H. armigera* colonies, one susceptible to the Vip3Aa toxin from *Bacillus thuringiensis* and the other selected for resistance, have been tested for two steps in the mode of action: binding to midgut receptors and activation by midgut juice. Competition-binding experiments were performed with brush border membrane vesicles prepared from larval midguts and ¹²⁵I-labeled Vip3Aa. Specific binding was found with vesicles of insects from both colonies, with similar binding parameters in both. Nevertheless, differences were found at the level of Vip3Aa

protoxin activation. Soluble proteinase extracts from resistant insects were 30% less active in the protoxin processing than extracts from susceptible insects, suggesting that an impaired protoxin activation is contributing to resistance to Vip3Aa in the selected insects.

POSTER/BACTERIA Wednesday 16:30 **BA-18**

Proteolytic processing of *Bacillus thuringiensis* Cry3 proteins by gut proteases and binding to brush border membrane vesicles from *Cylas puncticollis* (Brentidae)

Natalia M. Vera-Velasco, Patricia Hernández-Martínez, Baltasar Escriche

Departamento de Genética, Facultad de CC. Biológicas, Universitat de València, Spain

Correspondence: baltasar.escriche@uv.es

Bacillus thuringiensis Cry3Aa and Cry3Ca proteins have been reported to be toxic against the African sweet potato pest *Cylas puncticollis*. However, relatively little is known about the processing and binding interactions of these two Cry proteins. Proteolytic processing of Cry3Aa and Cry3Ca by the larval gut fluid of *C. puncticollis* was studied to characterize the gut proteases involved in the proteolytic processing. Moreover, we determined whether Cry3Aa and Cry3Ca proteins have shared binding sites in *C. puncticollis* brush border vesicles (BBMV). Processing of the Cry3Aa or Cry3Ca protoxins using either the gut fluid from *C. puncticollis* or commercial trypsin or chymotrypsin rendered similar fragments of about 55 kDa and 53 kDa, respectively. A serine-like protease from *C. puncticollis* gut fluid was found to be involved in the proteolytic processing of both Cry3 proteins. Homologous binding assays showed specific binding for the two Cry3 proteins on BBMV from *C. puncticollis* larvae. Heterologous competition assays showed that Cry3Aa and Cry3Ca partially competed for the same binding sites, suggesting that these proteins may have two different binding sites and only one of them is shared. Hence, our results suggest that pest resistance mediated by alteration of the shared Cry-receptor binding site might not render both Cry proteins ineffective.

POSTER Wednesday 16:30 **BA-19**

Domain III of *Bacillus thuringiensis* Cry1Ie toxin contributes to its binding to peritrophic membrane of Asian corn borer

Dongmei Feng, Zhen Chen, Nan Zhang, Chunlu Zhang, Shuyuan Guo

School of Life Science, Beijing Institute of Technology, Beijing 100081, China

Correspondence: guosy@bit.edu.cn

The insecticidal IE648 toxin is a truncated Cry1Ie protein with increased toxicity against Asian corn borer (ACB). Cry toxins form pores in the apical membrane of midgut cells. However the peritrophic membrane (PM) is an important barrier that Cry toxins must cross before binding to midgut cells. PM is a semipermeable matrix composed of chitin fibrils and protein-glycojugates that protect the insect gut epithelial cells from injury and infection. Previously it was shown that Cry toxins are able to bind and to accumulate in the PM of various lepidopteran insects. In this work we analyzed the binding interaction of IE648 to the PM of ACB, which showed to be specific by homologous competition binding assay. Heterologous competition binding assays performed with different fragments of domain I, domain II and domain III allowed us to identify that not domain I, but domain III of IE648 contributes to the interaction with PM, situation of domain II remains unclear. Furthermore, ligand blot assays indicated that IE648 and its domain III interact with chitin and with similar PM

proteins. These data provide a primary direction to understand the mechanism of interaction between PM and 3D-Cry toxin.

POSTER Wednesday 16:30 **BA-20-STU**

Loop regions of domain II of Cry1Aa have an important role for binding with BmABCC2 and its BmABCC2-depending activity

Satomi Adegawa¹, Shiho Tanaka^{1,2}, Shingo Kikuta¹, Ryoichi Sato¹

¹Graduate School of Bio-Applications and Systems Engineering

Tokyo University of Agriculture and Technology, Koganei, Tokyo,

Japan; ²Japan Society for the Promotion of Science Research

Fellowship for Young Scientists

Correspondence: ryoichi@cc.tuat.ac.jp

Cry toxins, insecticidal proteins produced by the bacterium *Bacillus thuringiensis*, have different insecticidal spectra in association with assigned subclass. This insecticidal specificity is thought to depend on the specific interaction between Cry toxins and insect's receptors. Recently, mutations of ABC transporter C2(ABCC2) were reported that they give insects high resistance to Cry1 toxins and this molecule have a very important role in the mode of action of Cry1 toxins. Cytotoxicity of Cry1 toxin mediated by ABCC2 is necessary to be elucidated. Our previous research suggested that difference in the structure of domain II of Cry1A strongly affected its cell-damaging activity in *B. mori* ABCC2(*BmABCC2*). In this study, we performed detailed analysis to elucidate which regions in domain II of Cry1Aa played an important role for binding with *BmABCC2* and its *BmABCC2*-dependent activity. We have some Cry1Aa mutants which have 4 amino acids replacements in domain II loop regions. Results from an *in vitro* toxicity assay using these mutants and *BmABCC2*-expressing Sf9 cells suggested some of the mutants have lower cell-damaging activity compared with wild-type toxin. Furthermore, we evaluated binding affinity between these mutants and *BmABCC2* by BIACORE system. In many mutants, there was some correlation between decrease in cell-damaging activity and decrease in binding affinity, but there was also some strongly conflicting results. These results suggested domain II of Cry1Aa is a region which affected its binding with *BmABCC2* but sometimes decreased in cell-damaging activity without affecting the binding. We will also discuss about what we are intending to do.

POSTER Wednesday 16:30 **BA-21**

sRNA mediated Cry toxin gene silencing helps *Bacillus thuringiensis* evading nematode feeding cessation defense behavior

Xiaoxia Luo[#], Donghai Peng[#], Ni Zhang, Suxia Guo, Jinshui Zheng, Ling Chen, Jian Lin, Lijiang Ruan, Ming Sun

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

[#]These authors contributed equally to this work.

Corresponding: m98sun@mail.hzau.edu.cn

Bacillus thuringiensis YBT-1518 is high toxic to nematodes which has three nematocides cry genes, *cry55Aa*, *cry6Aa*, and *cry5Ba*. Our previous work showed *Cry5Ba* is silent in YBT-1518. Here we revealed the mechanisms and ecological significance of *Cry5B* expression silence in *B. thuringiensis*. We confirmed *cry5B* silencing is not caused by *cry55Aa* and *cry6Aa* competition, and the flank sequence regulation. Western blots and RT-PCR showed *cry5Ba* silencing happened at post-transcriptional level. We then focused a potential small RNA BMBsr1. When co-expressed in cis- or trans- in YBT-1518, BMBsr1 inhibited the expression of *cry5Ba*, suggesting BMBsr1 negative regulates the expression of *cry5Ba*.

Using *gfp* and *lacZ* as report genes, we showed that BMBsr1 acts the RBS site of *cry5Ba*. Then we mapped the key action sites of BMBsr1 and showed the +5, +7, +13 sites are required for BMBsr1 mediated *cry5Ba* silencing. Finally, we found *Cry5B* does express *in vivo* after YBT-1518 was ingested by nematode *C. elegans*, and YBT-1518 benefits from *cry5B* silencing in colonization *in vivo*. This phenotype may help *B. thuringiensis* to cheat nematode host to feed its spores and crystals, and then express the toxin *in vivo* to kill the host. This work provides a special expression and regulation model for pathogen interaction with its host.

POSTER Wednesday 16:30 **BA-22**

Characterization of *Wolbachia* endosymbiont of *Lygus lineolaris*
Omaththage P. Perera, Gordon L. Snodgrass, Randall G. Luttrell

Southern Insect Management Research Unit, USDA-ARS,
Stoneville, MS 38776

Correspondence: op.perera@ars.usda.gov

Obligatory endosymbiotic Proteobacteria of the family Rickettsiaceae have been documented in many insect species. The tarnished plant bug, *Lygus lineolaris*, collected from field locations in the Mississippi Delta were screened using conserved multi locus sequence typing (MLST) PCR primers. At least three distinct strains (arbitrarily named *wLin1*, *wLin2*, and *wLin3*) of *Wolbachia* were identified in *Lygus* populations. All insects evaluated contained a single strain of *wLin* except for one individual infected with two strains. Polyclonal antibodies developed against the *Wolbachia* surface protein (WSP) identified *wLin* in developing eggs and the cells of ovaries of adult females. Dot blots of *Lygus* genomic DNA probed with the WSP gene indicated that approximately 60% of the *Lygus* in field populations are infected with a *wLin* strain. Impacts of *wLin* infections on the reproduction of *L. lineolaris* is under investigation.

POSTER Wednesday 16:30 **BA-23**

Biochemical characterization of parasporin-4 and effects of the pro-parasporin-4 diet on the health of mice

Shiro Okumura¹, Hironori Koga², Kuniyo Inouye^{3,4}, Eichichi Mizuki¹

¹Biotechnology and Food Research Institute, Fukuoka Industrial Technology Center, Fukuoka, Japan; ²Division of

Gastroenterology, Department of Medicine, Kurume University School of Medicine, Fukuoka, Japan; ³Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Kyoto, Japan; ⁴Inouye Laboratory of Enzyme Chemistry (iLEC), Kyoto, Japan

Correspondence: sokumura@fitc.pref.fukuoka.jp

Parasporin is a type of parasporal protein produced by *Bacillus thuringiensis* (Bt) that is capable of preferentially killing cancer cells. Bt strain A1470 produces pro-parasporin-4 (proPS4) with a molecular mass of 31 kDa, and it could be processed to parasporin-4 (PS4) the active form of it with that of 27 kDa by proteinase. PS4 is an aerolysin-type β -pore-forming toxin and it exhibits specific cytotoxicity against human cancer cell lines, CACO-2, Sawano, and MOLT-4 cells, although it did not exhibit insecticidal or hemolytic activities. The proPS4 could be solubilized in a weak acid solution e.g. 10 mM HCl and activated by pepsin. Most Bt toxins including parasporins are inactive in an acidic conditions, however, PS4 retains its cytotoxic activity in low pH conditions. That means the orally administered proPS4 could be solubilized and activated in the stomach of mammals. In this study, we examined the toxicity of parasporin-4 on ICR mice and also the influence of the oral administration of proPS4 against C57BL/6J mice. The EC₅₀ value of PS4 was 160 μ g/kg. Injection of PS4 induced decreases of the concentrations of some cations in

the urine and increases of the concentrations of creatinine and urea nitrogen in the serum, and that implies an injection of PS4 would impair the kidney functions of the mice. After an oral administration of proPS4, active PS4 was detected in the digestive tract of the mice, however, any serious health-hazard was not observed on the mice.

Diseases of Beneficial Invertebrates

POSTER Wednesday 16:30 **DB-1-STU**

Chitin-degrading protein PICBP49 - a key virulence factor of *Paenibacillus larvae*

Henriette Knispel¹, Anne Fünfhaus¹, Eva Garcia-Gonzalez¹, Lena Poppinga¹, Jennifer S M Loose², Gustav Vaaje-Kolstad², Elke Genersch¹

¹Institute for Bee Research, Hohen Neuendorf, Division of Molecular Microbiology and Bee Pathology

Friedrich-Engels-Str.32, D-16540 Hohen Neuendorf, Germany;

²Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, P.O. Box 5003, N-1432 Ås, Norway

Correspondence: elke.genersch@rz.hu-berlin.de

American Foulbrood (AFB) of honey bees is a globally occurring epizootic, caused by the gram-positive bacterium *Paenibacillus larvae*. Although AFB has been known for over hundred years, cellular and molecular details of pathogen-host interactions during pathogenesis of AFB remain poorly understood. However, the chitinolytic protein PICBP49 was recently identified as a key virulence factor during *P. larvae* infections. PICBP49 is responsible for degradation of the chitin-rich peritrophic matrix, a protective barrier in the larval gut. *In silico* analyses revealed a module belonging to the auxiliary activity 10 (AA 10) family of lytic polysaccharide monoxygenases (LPMOs), predicting PICBP49 to putatively degrade chitin via a special

POSTER Wednesday 16:30 **DB-2-STU**

Characterization of virulence factors in the fatal honey bee disease American foulbrood caused by *Paenibacillus larvae*

Julia Ebeling, Lena Poppinga, Anne Fünfhaus, Elke Genersch
Institute for Bee Research, Hohen Neuendorf, Brandenburg, Germany

Correspondence: elke.genersch@rz.hu-berlin.de

American foulbrood is a very contagious, worldwide occurring honey bee brood disease caused by the gram-positive, spore-forming bacterium *Paenibacillus larvae*. The larvae ingest the bacterial spores together with their regular food. The spores germinate in the larval midgut, where the vegetative cells proliferate until the midgut is massively filled. Thereafter, it comes to the breaching of the peritrophic matrix and the midgut epithelium. Thereupon, the bacteria are free to invade the host's hemocoel followed by larval death. Interestingly, four *P. larvae* genotypes exist which are classified by enterobacterial repetitive intergenic consensus polymerase chain reaction, and accordingly named ERIC I-IV. The most relevant genotypes being isolated from current American foulbrood outbreaks are ERIC I and II. The genotypes also differ phenotypically, and most importantly have developed different infection strategies. Recently, two binary AB toxins, Plx1 and Plx2, have been identified as virulence factors in the genotype ERIC I. Contrarily in ERIC II, the S-layer protein SpiA is involved in pathogenesis. Previous studies predict these proteins to play a role in the breakthrough of the epithelial cell

barrier. Moreover, the chitin-binding protein PICBP49 acts as common virulence factor shared by both genotypes, and is involved in the degradation of the peritrophic matrix. We aim at further analyzing and characterizing the familiar virulence factors of *P. larvae* and at the identification of new candidates to further understand the infection mechanism of this dangerous honey bee pathogen.

POSTER Wednesday 16:30 **DB-3**

Decentralised molecular diagnostics and remote data reporting for management of disease in global aquaculture

Kelly S. Bateman¹, Michelle Pond¹, Peter Munday², Olga Gandelman², Grant D. Stentiford¹

¹European Union Reference Laboratory for Crustacean Diseases, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Barrack Road, The Nothe, Weymouth, Dorset DT4 8UB, UK; ²Epistem, Genedrive, Epistem Ltd., 48 Grafton Street, Manchester, M13 9XX, UK

Correspondence: kelly.bateman@cefas.co.uk

Disease is widely acknowledged as the prominent bottle-neck to achieving global food security and poverty alleviation targets relating to aquaculture with annual losses exceeding US \$6bn. High profile disease in \$15bn shrimp industry include those caused by White Spot Syndrome Virus (WSSV), the bacterial pathogen implicated in Acute Hepatopancreatic Necrosis Disease (AHPND) and emergent pathogens such as *Enterocytozoon hepatopenaei*, implicated in Early Mortality Syndrome (EMS); these pathogens implicated in annual losses of \$3bn per annum. Genedrive[®] is a small footprint molecular diagnostics platform capable of rapid, sensitive and specific detection of pathogens within an hour. It combines proprietary 'hybrid' thermal engine technology with bespoke consumable elements designed for detection of the pathogen/s of interest. An ultra-simple, 'single-button' operation allows for the operation of the equipment by un-skilled operatives, with minimal training. Developed for use in human pathogen diagnostics (currently tuberculosis testing in Africa), the technology has high potential for accurate detection of pathogens in other settings where rapid detection is required and where centralised laboratory infrastructure is poor. We are currently working to test and validate Genedrive[®] against gold standard diagnostics for WSSV and AHPND applied to penaeid shrimps. In addition, we are developing a bespoke smartphone app to interface with Genedrive[®] and to transmit field data to a centralised data repository for subsequent analysis. The formation of an accurate, low-cost diagnostic and integration with user-technology reporting of data has the potential to revolutionise disease management in global aquaculture and will contribute directly to poverty alleviation and global food security associated with aquaculture.

POSTER Wednesday 16:30 **DB-4**

Virus prevalence in bee populations in apple orchards in New York, USA

John P. Burand¹, Shuning Zheng², Bryan N. Danforth³

¹Department of Microbiology; ²Graduate Program in Molecular and Cellular Biology, University of Massachusetts - Amherst, Amherst, MA 01003, USA; ³Department of Entomology Cornell University, Ithaca, NY 14853 USA

Correspondence: jburand@microbio.umass.edu

We examined the prevalence of Black Queen Cell Virus (BQCV), and Deformed Wing Virus (DWV) and Sacbrood Virus (SBV) in *Apis* (Apidae) and *Andrena* (Andrenidae) bee pollinators found foraging on apple in five orchards in Central New York State. While *Apis* is a

social bee found primarily in managed hives, *Andrena* are solitary, ground-nesting bees. Bees were collected in mid-May and included approximately 24 bees of each group when available. BQCV was the most prevalent of the three viruses with 71% of the *Apis* and 49% of the *Andrena* testing positive for this virus. DWV was found in 38% of *Apis* and 29% of the *Andrena* collected from these sites. The prevalence of SBV was 29% and 6% in *Apis* and *Andrena* respectively. For all orchards virus levels were higher in *Apis* than *Andrena* except at two sample sites where BQCV was more prevalent in *Andrena*. We are currently examining BQCV gene sequences to determine the relationship between the viruses amplified from bees of these two different families. This is the first reported survey of honey bee viruses in native, solitary andrenid bees.

POSTER Wednesday 16:30 **DB-5**

Insect pathological challenges in insects produced for food and feed

Jørgen Eilenberg¹, Christina Nielsen-LeRoux², Silvia Cappellozza³, Just M Vlak⁴, Annette B Jensen¹

¹University of Copenhagen, Department of Plant and Environmental Sciences, Copenhagen, Denmark; ²INRA, UMR 1319 Micalis-AgroParisTech, France; ³Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Honeybee and Silkworm Research Unit, Padova, Italy; ⁴Wageningen University, Laboratory of Virology, Wageningen, the Netherlands

Corresponding author: jei@plen.ku.dk

Over the latest years, there has been a significant increase in the production of insects for food and feed. Increased production of insects at large scale will lead to many novel challenges, including problems with diseases, similar to what is seen in any other large scale production system. In addition, it is to be expected that new and hitherto neglected or even unknown diseases will emerge in insect production systems. Honeybee and silkworm are mostly produced for other reasons than as human food, yet we can use them as examples to learn about emergence over time of new diseases in production insects. Insect pathologist are needed for disease diagnostics and to develop guidelines for disease control. Contact and collaboration with commercial producers is essential. We provide an overview of insect diseases in the most commonly produced insect species for food and feed, based on a 2014 questionnaire sent to commercial producers.

POSTER Wednesday 16:30 **DB-6-STU**

The influences of insect-mediated mental healthcare program to children with mental disease

Seung Hee Lee¹, Sung Min Bae¹, Young Soon Jun², Tae Young Shin¹, Yong Oh Ahn¹, Won Seok Gwak¹, Soo Dong Woo¹

¹Department of Agricultural Biology, College of Agriculture, Life & Environment Science, Chungbuk National University, Cheongju 361-763, Korea; ²Department of Neuropsychiatry, Konkuk University Chungju Hospital, Chungju 380-704, Korea

Correspondence: sdwoo@cbnu.ac.kr

Insects are among the most diverse groups of animals on the planet, representing more than half of all known living organisms. These insects are found in nearly every environment. Although humans regard certain insects as pests and attempt to control them using insecticides, most insects perform complex ecological functions, and provide either direct or indirect economic benefits to humans. Recently, the importance of insects used as food sources or as pets has increased in many countries, including Korea. In addition, several insects have a strong influence on people's emotion. Insect-mediated mental healthcare program is

designed to help people who have disorders with physical, behavior and development. Children who have mental disorder, the experimental group that was provided with an insect-mediated mental healthcare program over a total of 8 sections, one section per week, 60 minutes per section, followed by pre-test and post-test. They responded to therapeutic effect after the completion of the program. Further research on the basis of this study is expected to help children with emotional therapy in other areas.

metal ion-dependent oxidative mechanism. This work aims to further elucidate the important role of *PICBP49* during *P. larvae* infections by characterize this protein biochemically. After successful expression of *PICBP49* in *E. coli*, a purification protocol could be acquired. With the further help of affinity assays, mass spectrometry and chromatography we analyzed enzyme capacities, e.g. chitin degrading abilities, substrate preferences and binding constants as well as product profiles and rates. Incubation assays of isolated peritrophic matrices of honey bee larvae and other species were performed to proof the generated concept of separated degradation experiments *in vitro*.

POSTER Wednesday 16:30 **DB-7**

Development and efficacy of insect-mediated mental healthcare program for the public

Sung Min Bae¹, Young Soon Jun², Tae Young Shin¹, Won Seok Gwak¹, Yong Oh Ahn¹, Seung Hee Lee¹, Soo Dong Woo¹

¹Department of Agricultural Biology, College of Agriculture, Life & Environment Science, Chungbuk National University, Cheongju 361-763, Korea; ²Department of Neuropsychiatry, Konkuk University Chungju Hospital, Chungju 380-704, Korea
Correspondence: sdwoo@cbnu.ac.kr

Mental health is a great concern to people in modern times. Mental health includes subjective well-being, perceived self-efficacy, autonomy, competence, intergenerational dependence, and self-actualization of one's intellectual and emotional potential. Maintaining good mental health is crucial to living a long and healthy life; this has become increasingly clear in recent years. Various therapies have been developed to prevent mental health problems, including music therapy, art therapy, play therapy, drama therapy, horticultural therapy, and animal-assisted therapy. Recently, the importance of insects used as food sources or as pets has increased in many countries, including Korea. In addition, several insects have a strong influence on people's emotion. In this study, we developed the insect-mediated mental healthcare program for the public and evaluated its efficacy. This program was divided into beginning, middle and final parts that were subdivided into six sections, one section per week, 60 minutes per section, followed by pre-test and post-test. They increased to self-esteem, subjective quality of life and quality of life after the completion of the program. Further research on the basis of this study is expected to help public with emotional therapy in other areas.

POSTER Wednesday 16:30 **DB-8-STU**

Efficacy of insect-mediated mental healthcare program to an adolescent

Won Seok Gwak¹, Tae Young Shin¹, Young Soon Jun², Sung Min Bae¹, Yong Oh Ahn¹, Seung Hee Lee¹, Soo Dong Woo¹

¹Department of Agricultural Biology, College of Agriculture, Life & Environment Science, Chungbuk National University, Cheongju 361-763, Korea; ²Department of Neuropsychiatry, Konkuk University Chungju Hospital, Chungju 380-704, Korea
Correspondence: sdwoo@cbnu.ac.kr

Adolescence is a critical period of rapid growth physically, emotionally, mentally and for the development of personality. These students are the real workhorses of the society, and they have the added stress of struggling to get noticed so they can move up in the society one day. Therefore, the goal of our study was to develop of healthcare program using rearing of insects and making of handicraft relation insects, these are limelight in the world. In addition, we attended to develop our own insect-mediated program to middle students and evaluate their psychological healing effects after program. The insect-mediated program was performed once a week for 2 months. The each session did not over 50 min and psychological healing effects were investigated by psychological test paper. As results, the participated students at insect-mediated program increased their mental health including a self-worth to rearing of insects and making of handicraft relation insects. These results may be helpful to develop insect-mediated mental healthcare programs.

Fungi

POSTER Wednesday 16:30 **FU-1**

ITS region analysis of isolates of *Beauveria bassiana*, a pathogenic fungus to the silkworm, *Bombyx mori* L.

Lianqen Shi, Jie Jin

College of Animal Sciences, Zhejiang University, Hangzhou, Zhejiang, P.R.China

Correspondence: slgsilk@zju.edu.cn

ITS1-5.8S rDNA-ITS2 region of 21 isolates of *Beauveria bassiana*, a pathogenic fungus to the silkworm, *Bombyx mori* were amplified and sequenced. The analysis of all aligned sequences by Mega 3.0 produced a most parsimonious phylogenetic tree. The ITS region of the *Beauveria bassiana* isolates from Nanning (Bb03), Zunyi (Bb06) and Mengzi (Bb07) are significant differences with other isolates, but were 100% homology to the sequence of *Cordyceps bassiana*, teleomorph of *Beauveria bassiana* by submitted to GenBank in National Center for Biotechnology Information (NCBI) through accession number AB027382. There are many sites of mutation in the *Beauveria bassiana* isolates from Yongji (Bb20) and Yantai (Bb21), but were deletion or mutation of base pair of only a few points in other isolates. ITS region was relatively conservative within species level, was no significant differences among isolates. So, the ITS region analysis was very reliable to classification and identification between species of *Beauveria bassiana*, a pathogenic fungus to the silkworm, *Bombyx mori*.

POSTER Wednesday 16:30 **FU-2**

Molecular characterization of *Isaria fumosorosea* from citrus and strawberry crops in Brazil

Celeste P. D'Alessandro¹, Ricardo Macedo do Nascimento¹, Gustavo Maruyama Mori^{2,3}, Maria I. Zucchi³, Italo Delalibera Junior¹

¹Department of Entomology and Acarology, "Luiz de Queiroz" College of Agriculture (ESALQ), University of São Paulo (USP), Piracicaba, São Paulo, Brazil; ²Center for Molecular Biology and Genetic Engineering, University of Campinas, Campinas, São Paulo, Brazil; ³São Paulo Agency for Agribusiness Technology, Piracicaba, São Paulo, Brazil

Correspondence: celed1881@gmail.com

The entomopathogenic fungus *Isaria fumosorosea* (Ascomycota: Hypocreales: Cordycipitaceae) is a natural soil habitant and infects several species of arthropods. This fungus has been used for the

biological control of several important agricultural pest insects and the genetic diversity knowledge would substantially add for its rational use as a biological control agent. The aim of this study was to characterize the genetic diversity of *I. fumosorosea* strains from soil, rhizosphere and insects found on citrus and strawberry crops and from native vegetation in Brazil. We evaluated 44 isolates and their genetic variability was investigated using eight microsatellite loci and amplified fragment length polymorphism (AFLP). For microsatellite markers, we observed a substantially low genetic diversity by means of Nei's gene diversity, allelic richness or Shannon-Weiner diversity index. Regarding the AFLP marker, we performed 14 combinations of selective primers and 200 polymorphic fragments ranging from 50 to 350pb were yielded. Nei's gene diversity was also relatively low (0.25), but we did not detect clones considering 3% of genotyping errors. Using the Bayesian clustering method implemented in the Structure software, the genetic variation of the 44 isolates was clustered in two main groups. Furthermore, the analysis of molecular variance (AMOVA) showed that the grouping that retained the maximum variation corresponded to the grouping based on the fungi source (soil or insect), not on crops nor on geographic origin. These results, besides being scientifically interesting from a microevolutionary perspective, will be useful for properly managing *I. fumosorosa* as a biological control agent.

POSTER Wednesday 16:30 **FU-3-STU**

Comparative genomics of cold-adapted *Metarhizium frigidum*

Brian Lovett, Raymond St. Leger

University of Maryland, College Park

Correspondence: lovettbr@umd.edu

The genomes of entomopathogenic fungi are being sequenced at an impressive rate. Thus far, these include over seven high-quality genomes of *Metarhizium* spp. that illuminate the unique biology of these fungi, and the enigmatic history behind their divergence as specialist or generalist insect pathogens that are also multifactorial plant growth promoters. Here, I present the finished sequence for *Metarhizium frigidum*, a cold-adapted member of the *Metarhizium* genus. Using a bioinformatics approach, we report the full annotation of the genome with analysis of protein family enrichment and other notable genomic features of this unique *Metarhizium* species. Apart from bolstering the ranks of sequenced *Metarhizium* fungi, this fungus represents an opportunity to examine cold adaptation in fungi, and in particular, *M. frigidum*'s ability to maintain pathogenicity at low temperatures. This in turn can be applied to efforts to use entomopathogenic fungi as biofertilizers and in biocontrol. Since *Metarhizium* fungi have emerged as model organisms for studying host specificity and plant associations, the results will include characterization of genetic information associated with these lifestyles.

POSTER/ Wednesday 16:30 **FU-4**

Identification of *Drosophila* mutants affecting defense to an entomopathogenic fungus

Hsiao-Ling Lu, Jonathan Wang, Markus Brown, Chris Euerle, Raymond St. Leger

Entomology Department, University of Maryland, College Park, MD, USA

Correspondence: jonwang@umd.edu

Fungi cause the majority of insect disease. However, to date attempts to model host-fungal interactions with *Drosophila* have focused on opportunistic human pathogens. Here, we performed a screen of 2,613 mutant *Drosophila* lines to identify host genes

affecting susceptibility to the natural insect pathogen *Metarhizium anisopliae* (Ma549). Overall, 241 (9.22%) mutant lines had altered resistance to Ma549. Life spans ranged from 3.0 to 6.2 days, with mutations increasing susceptibility being 1.6-fold commoner than mutations increasing resistance, and females being more susceptible than males in all lines. Speed of kill correlated with within-host growth and onset of sporulation, but slow kill did not reduce total spore production indicating that this measure of fitness is decoupled from host genotypes. Results showed that mutations affected the ability of *Drosophila* to restrain rather than tolerate infections and suggested trade-offs between antifungal and antibacterial genes affecting cuticle and gut structural barriers. Approximately, 13% of mutations were in genes previously associated with host pathogen interactions. These encoded fast-acting immune responses including coagulation, phagocytosis, encapsulation and melanization but not the slow-response induction of anti-fungal peptides. The non-immune genes impact a wide variety of biological functions, including behavioral traits; over 62% of mutations have pleiotropic interactions with starvation stress. Many have human orthologs already implicated in human disorders; while others were mutations in protein and non-protein coding genes for which disease resistance was the first biological annotation.

POSTER Wednesday 16:30 **FU-5-STU**

Persistence of two Brazilian isolates of *Metarhizium* in a strawberry cropping system using microsatellite markers

Thiago Rodrigues de Castro^{1,2}, Johanna Mayerhofer³, Jürg Enkerli³, Jørgen Eilenberg², Nicolai V. Meyling², Italo Delalibera Jr.¹

¹University of São Paulo (ESALQ), Brazil; ²Department of Plant and Environmental Sciences, University of Copenhagen, Denmark;

³Institute for Sustainability Sciences, Agroscope, Switzerland

Correspondence: castrotr@gmail.com

The aim of this study was to evaluate the persistence of two Brazilian isolates of *Metarhizium* (*M. anisopliae*-ESALQ1037- and *M. robertsii*-ESALQ1426) that were applied in a strawberry crop in Minas Gerais state in Brazil. The application was made as a randomized block set (10 blocks with 3 treatments), applying 4×10^4 conidia/ha of the isolates on the surface of the soil near the root of the plants in each plot. The control plot was treated with water only. Soil samples were collected once before the application (September 2012) and 3 times after (January, April and August 2013), and one root sampling was performed in August 2013. Two methods, i.e., insect baiting and plating soil suspensions on a selective medium, were used to isolate *Metarhizium* spp. To determine the different genotypes, fifteen simple sequence repeat (SSR or microsatellite) markers were PCR amplified from each of the 138 isolates. The EF1- α and MzFG543igs of one representative isolate per genotype was sequenced to determine species affiliation. Twenty six percent and 22% of the obtained isolates were identified as *M. robertsii*-ESALQ1426 and *M. anisopliae*-ESALQ1037. Of the remaining 52% of the isolates, 42% belonged to two indigenous *M. robertsii* clades, 8% to *M. anisopliae* and 1% each to *M. pingshaense* and *M. brunneum*. Results have shown that the two applied isolates persisted in the soil for at least one year after application. Furthermore, results indicated that, *M. robertsii* is better adapted to the experimental field than other *Metarhizium* spp., since a) it was found to occur naturally, and b) the applied *M. robertsii* strain was re-isolated at a higher (2,24 times) rate than the applied *M. anisopliae* isolate.

*IMBICONT "Improved biological control for IPM in fruits and berries" (Brazil/FAPESP:2011/51556-3 and Denmark/FP7-ENV-2011-ECO-INNOVATION:282767-2)

POSTER Wednesday 16:30 **FU-6**

Susceptibility of biocontrol fungi in the genera *Nomuraea*, *Isaria*, *Purpureocillium*, *Pochonia*, and *Trichoderma* to imbibitional damage and its mitigation through increased conidial quality
 Marcos Faria, Irene Martins, Daniela A. Souza, Rogério B. Lopes
 EMBRAPA Genetic Resources and Biotechnology, Brasília, DF, Brazil

Correspondence: marcos.faria@embrapa.br

When dried conidia are plunged into cold water, rapid imbibition may cause cell death. We assessed the susceptibility to this imbibitional damage (ID) of two or three isolates for each of the seven species or varieties of biocontrol fungi considered in this study. A commercial *Metarhizium anisopliae* isolate, known to be sensitive to ID, was also included. All *Isaria farinosa* isolates showed significant viability losses (19.1-93.0%) due to drying of conidia with a desiccant, prior to immersion in water. For other isolates, losses in this step were in the 0-12.7% range. Dried *M. anisopliae* conidia were the most sensitive to ID, with a 41% germination drop following immersion in cold water (15 °C) when compared to immersion in warm water (37 °C). For some *I. farinosa*, *I. fumosorosea* and *Trichoderma asperellum* isolates, and all isolates belonging to *Purpureocillium lilacinum* and *Pochonia chlamydosporia* (varieties *catenulata* e *chlamydosporia*), losses varied from 15.3 to 38.5%. Germination losses for remaining isolates (including two *Nomuraea rileyi* isolates produced on agar medium) were in the 0-5.2% range. Complementary studies with dehydrated *M. anisopliae* conidia produced on different substrates revealed that low sensitivity to ID is directly correlated with elevated conidial vigor following dehydration. Low mortalities (0-7%) of dehydrated conidia plunged into cold water were seen when post-drying vigor was $\geq 95\%$ (i.e., 95% of conidia plated on PDA were capable of germinating within 22 h following incubation at 25 °C), regardless of agar media or cooked cereal used for production of propagules.

POSTER Wednesday 16:30 **FU-7-STU**

Light during mycelial growth induce increased tolerance of conidia to different types of stress in entomopathogenic fungi

Luciana P. Dias^{1,2}, Claudinéia A. S. Araújo¹, Paulo C. Ferreira², Breno Pupin¹, Drauzio E. N. Rangel¹

¹Instituto de Pesquisa e Desenvolvimento, Universidade do Vale do Paraíba, São José dos Campos, SP 12244-000, Brazil; ²Escola de Engenharia de Lorena (EEL/USP)/ Pós-Graduação em Biotecnologia Industrial, Estrada Municipal do Campinho, Lorena, SP, Brazil

Correspondence: lucianapereiradias@live.com

The effect of visible-light exposure during mycelial growth was investigated on the conidial tolerance of ten insect-pathogenic fungi to: UV radiation (A), osmotic stress caused by potassium chloride (B), and genotoxic stress caused by 4-nitroquinoline1-oxide (C). Conidia of ten species were produced: 1) on potato dextrose agar medium (PDA) in the dark (control), 2) on PDA under continuous visible light, and 3) under nutritional stress (= Czapek medium without sucrose - MM) in the dark. Conidia of *Metarhizium robertsii* (ARSEF 2575) and *Isaria fumosorosea* (ARSEF 3889) produced under visible light had higher tolerance to (A). Five species [*Beauveria bassiana* (ARSEF 252), *M. brunneum* (ARSEF 1187), *M. robertsii* (ARSEF 2575), *Tolypocladium cylindrosporium* (ARSEF 3392), and *Aschersonia aleyrodalis* (ARSEF 10276)] were more tolerant to (B). Three species [*M. brunneum* (ARSEF 1187), *M. anisopliae* (ARSEF 5749), and *A. aleyrodalis* (ARSEF 10276)] become more tolerant to (C). The mycelial growth under MM significantly induced increased conidial tolerance to all three

stress conditions for the species *B. bassiana* (ARSEF 252), and *T. inflatum* (ARSEF 4877). MM induced higher tolerance of *M. brunneum* (ARSEF 1187), *M. robertsii* (ARSEF 2575), and *M. anisopliae* (ARSEF 5749) to (A) and (B), but not to (C). *T. cylindrosporium* (ARSEF 3392) grown on MM was more tolerant to (A) only. The only species that did not respond either to light or nutritional stress were *Lecanicillium aphanocladii* (ARSEF 6433) and *Simplicillium lanosoniveum* (ARSEF 6651). Visible light is, therefore, an important factor that induces stress tolerance in some insectpathogenic fungi.

POSTER Wednesday 16:30 **FU-8**

The great soil-sampling survey: A Utah State University/USDA collaborative project to find new entomopathogenic fungi from western USA soil

Chad A. Keyser, Samuel P. Sherwood, Everton K. K. Fernandes, Carmina Moore, Patricia S. Golo, JoAnn Stark, Alesandra M. Fernandes, Rodrigo B. Ferreira, Scott Treat, Fabiana Alvarez, Janitha B. Nandalochana, Holly C. Suisse, Stefanie Selamet, Laurel Nicolson, Skylar Christensen, R. Nelson Foster*, Lerry E. Jech*, Stefan T. Jaronski*, Donald W. Roberts

Department of Biology, Utah State University, Logan Utah, USA

*USDA, APHIS, PPQ, CPHST

Correspondence: chadaltontkeyser@gmail.com

The aim of this project was to isolate new entomopathogenic fungi from soil collected in western US states and to evaluate their potential as biological control agents for orthopteran pests. Over an eight year period 38,075 soil samples were collected from 17 states. Every soil sample was plated on selective media developed specifically for the project, which resulted in 2,433 isolations of *Metarhizium* spp., *Beauveria* spp. and other entomopathogenic fungi. Genus identification was performed using morphological features; species identity based on molecular genotyping for 491 of the *Metarhizium* isolates was performed using the α -elongation factor. A virulence screening of 736 *Metarhizium* isolates was conducted with a model host insect larvae (*Tenebrio molitor*); and 512 isolates were evaluated for tolerance to UV-B irradiation and 45°C heat exposure. All of the isolates have been deposited in the ARSEF fungal collection. The project has collaborated with both US and foreign laboratories and scientists, and 33 peer-reviewed publications have resulted thus far. Overall, these studies clearly show the diversity and heterogeneous distribution of entomopathogenic fungi in soil. Also, the variability between isolates in virulence and stress tolerances are clearly demonstrated; emphasizing the need to assess individually important biological control characteristics of each new isolate. Large-scale collaborative studies, such as this, have great potential to illuminate the composition, distribution and potential for natural pest suppression of entomopathogenic fungi; and hopefully, will lead to the discovery and development of more effective biological control agents.

POSTER Wednesday 16:30 **FU-9**

Comparison of the use of insect baiting methods versus selective media when determining the diversity of *Metarhizium* spp. in soil

Carmela Hernández-Domínguez, Ariel W. Guzmán-Franco

Postgrado en Fitosanidad-Entomología y Acarología, Colegio de Postgraduados, Km. 36.5 Carretera México-Tezcoco, Montecillo, Tezcoco, Estado de México, 56230, Mexico

Correspondence: gariel@colpos.mx

When developing a fungal-based biocontrol agent, it is important to assess the regional diversity of native entomopathogenic fungi

prior to release. The two methods commonly used for the detection of entomopathogenic fungi such as *Metarhizium* spp. in soil are (i) the use of *Galleria mellonella* larvae as a bait, and (ii) selective media. When used simultaneously these methods can provide different results and, therefore, their use is currently under discussion. We compared the accuracy of baiting with *G. mellonella* versus the use of two selective media (CTC and DOD) for the recovery of *M. anisopliae*, *M. robertsii*, *M. brunneum* and *M. pingshaense*. For this, we artificially inoculated soil with conidia from the four species to produce two concentrations (2×10^5 and 2×10^3 conidia gr^{-1}). Using selective media it was possible to identify significant differences amongst the four *Metarhizium* species, with *M. robertsii* producing most colony forming units (CFU) recovered followed by *M. anisopliae*, *M. pingshaense* and *M. brunneum*. More CFU's of *M. anisopliae*, *M. robertsii* were recovered using CTC media compared to DOD regardless of the conidia concentration whilst DOD was the optimal media for *M. brunneum* and *M. pingshaense*. More CFU were recovered at the higher concentration (2×10^3 conidia gr^{-1}). The results of baiting with *G. mellonella* showed a greater recovery of *M. anisopliae* followed by *M. brunneum*, *M. pingshaense* and *M. robertsii*, regardless of the conidia concentration. The possibility of combining both methods to obtain more accurate results are discussed.

POSTER Wednesday 16:30 **FU-10-STU**

Antifungal activity of entomopathogenic fungi isolated in Korea and their geographical characteristics

Yong Oh Ahn, Tae Young Shin, Sung Min Bae, Won Seok Gwak, Seung Hee Lee, Soo Dong Woo

Department of Agricultural Biology, College of Agriculture, Life & Environment Science, Chungbuk National University, Cheongju 361-763, Korea

Correspondence: sdwoo@cbnu.ac.kr

Entomopathogenic fungi are natural enemies of insects and contribute to the regulation of their host populations. Their mode of action against insects involves the attachment of conidia to the insect cuticle, followed by germination, cuticle penetration, and internal dissemination throughout the insect. During this process, secreted enzymes, protein toxins, and secondary metabolites can be used by the fungus to overcome the host immune system, modify host behavior, and defend host resources against competing pathogens and saprophytes. Additionally, these metabolites exhibit variety bioactivities, and they have been suggested as potential candidates for the development of new bioactive agents. In this regard, recently, most of studies searching for antifungal substances from fungi were performed in worldwide. However, the distribution and characterization of entomopathogenic fungi having antifungal activity is not reported yet to our knowledge. At present, 28 species and 20 genera of 342 entomopathogenic fungi from diverse natural habitats of South Korea soils have been isolated and identified in our laboratory. From our preliminary experiments, we have identified of fungi especially entomopathogenic fungi that have antibacterial, antioxidant and anticancer activities. The goal of our study was to establish a pool of entomopathogenic fungi having antifungal activity regard to habitat type. This study can be a little guide for future entomopathogenic fungal source programs and the selected fungal isolates having antifungal activity in this study may be useful as the source of antifungal substances.

POSTER Wednesday 16:30 **FU-11**

Characterization and pathogenicity of a *Beauveria pseudobassiana* strain that collapsed a laboratory colony of *Dendroctonus ponderosae* (Coleoptera: Scolytidae)

George Kyei-Poku¹, Debbie Gauthier¹, Fernando Mantouwane¹, Johny Shajahan², Will Fick¹

¹Natural Resources Canada, Canadian Forest Service, Great Lakes Forestry Centre Sault Ste. Marie, Ontario, Canada; ²Amco Farms Inc., Leamington, ON, Canada

Correspondence: gkveipok@nrcan.gc.ca

The mountain pine beetle (MPB), *Dendroctonus ponderosae* Hopkins is a well-entrenched insect pest to western Canada and the United States and causes severe disturbance regime by the ever range expansion in lodgepole and other pine forests. Control strategies which have been applied so far are still insufficient to prevent its damage and eastward spread. Entomopathogenic fungi which have shown potential to regulate other beetle pests as biological control agents could play a relevant role in the management of MPB. The *Beauveria* isolate (MPB-UBK) that caused the collapse of our lab MPB colony had been identified as a new member of the species *B. pseudobassiana* based on the partial EF1-alpha, intergenic Bloc and ITS sequences homology and concurrent phylogenetic analysis. Virulence of *B. pseudobassiana* - MPB-UBK isolate was compared with commercial agents *Beauveria bassiana* (Botanigard ES; Bb); *Metarhizium brunneum* (Met F52 ES; Mb); *Isaria fumosorosea* (PFR 97 20% WDG™; Ifr); nine highly pathogenic strains including *Beauveria bassiana* (2), *Beauveria pseudobassiana* (2), *Metarhizium robertsii* (2), and *Isaria fumosorosea* (1) on MPB adults under laboratory conditions.

All the *Beauveria* spp. isolates were found to be highly infectious and virulent against MPB. The *B. pseudobassiana* - MPB-UBK isolate caused approximately 90.0% mortality of MPB adults 6 days post inoculation in the concentration of 1×10^6 conidia/mL. Consequently, *B. pseudobassiana* - MPB-UBK appears to be a promising microbial control agent to exploit in the management of for biocontrol of *Dendroctonus ponderosae*.

POSTER Wednesday 16:30 **FU-12**

Entomopathogenic fungi for six species of leaf-cutting ants from different sites of Argentina

Daniela Goffré, Jorge A. Marfetan, Patricia J. Folgarait

Laboratorio de Hormigas, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Bernal, Buenos Aires, Argentina

Correspondence: patricia.folgarait@gmail.com

We investigated the entomopathogenic fungi associated with workers from 6 species of leaf-cutting ants (*Acromyrmex lundii*, *A. crassispinus*, *A. heyeri*, *A. lobicornis*, *A. aspersus* and *A. striatus*), in 8 sites located in 5 Phytogeographical Provinces (Pampeana, Espinal, Monte, Yungas and Patagónica) in Argentina. We hypothesized that, at least, the sampling site could explain similarities among entomopathogen's communities, mainly because of opportunistic horizontal transmission. From 3748 ants, we recorded 31.2% of ants infected with entomopathogenic fungi. *Fusarium oxysporum* and *F. solani* were the most widely distributed, followed by *Purpureocillium lilacinum*. Río Negro (Patagónica Province) exhibited the greatest richness with 13 species, followed by the site from Monte Province with 10 species. We advanced the hypothesis that *Penicillium* sp.1 and *C. equinulata* var *verticillata* could be considered indicator species of Río Negro and Salta (Yungas), respectively, as they were registered in most nests only from these sites. We found that *A. aspersus* located in Tucumán (Yungas) had more

entomopathogens (median: 59%) than other ant species and sites, followed by *A. lobicornis* (41%) from La Pampa (Monte). We run several cluster analyses with presence-absence of entomopathogens at different levels: nests, sites, phytogeographical provinces, and ant species. We found that only sites exhibited similarities among the entomopathogen's communities in agreement with our hypothesis. However, there were great differences among nests within sites probably due to the dissimilar health status or capacity to avoid infections.

POSTER Wednesday 16:30 **FU-13**

First isolation of *Beauveria* and *Metarhizium* from a wheat stem borer, *Cephus cinctus* (Hymenoptera: Cephidae) in North America

*Stefan T. Jaronski*¹, *Gadi V. P. Reddy*², *Shaohui Wu*², *John F. Gaskin*³, *Stephen Rehner*³

¹Agricultural Research Service, U.S. Department of Agriculture, Northern Plains Agricultural Research Laboratory, Sidney MT 50270 USA; ²Montana State University, Western Triangle Research Center, Conrad MT 59425 USA; ³Agricultural Research Service, U.S. Department of Agriculture, Systematic Mycology Laboratory, Beltsville MD 20705 USA

Correspondence: Stefan.Jaronski@ars.usda.gov

The wheat stem sawfly, *Cephus cinctus*, a grass-mining cephid attacking wild grasses and small grains, is the most important pest of wheat in the Plains region of the U.S. and southern Canada. Yield losses occur due to larval tunneling and stem lodging and annual losses are estimated at more than \$100 million just in MT. Except for the adult stage, entire development of *C. cinctus* takes place inside the plant. We unexpectedly encountered *Beauveria bassiana* s.l. infections in live, diapausing larvae in spring 2013. Subsequent surveys in autumn 2013 and 2014 yielded 61 additional *Beauveria* isolates, plus three isolates of *Metarhizium flavoviride* s.l. Analysis with *Bloc* gene sequence revealed the isolates fall into either Rehner's *B. bassiana* Clade A4, or *B. pseudobassiana*. Only a few of 50+ wheat fields sampled had *Beauveria* infections among the larvae from a site, but prevalence in positive fields was very high, reaching 100% in some fields. Overall prevalence was 1.1%. While there was strong clustering of genotypes within a wheat field, there was great overall diversity. Given the biology of the insect, totally isolated within the wheat pith, it is highly likely that these infections arose from endophytic *Beauveria* and *Metarhizium*. Several of the original isolates were found to be highly pathogenic to wheat stem sawfly larvae and work is underway to determine endophytic potential of several strains. Research reported here is being funded by multiple grants from the Montana Wheat and Barley Committee.

POSTER Wednesday 16:30 **FU-14**

First report of a mosquito-pathogenic *Leptolegnia* sp. (Saprolegniales) in Brazil

*Cristian Montalva*¹, *Richard A. Humber*², *Karine dos Santos*¹, *Stefanie Buchter*¹, *Karin Collier*³, *Luiz F.N. Rocha*^{1,4}, *Éverton K.K. Fernandes*¹, *Christian Luz*¹

¹Laboratório de Patologia de Invertebrados, Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, GO, Brasil; ²USDA-ARS Collection of Entomopathogenic Fungal Cultures, RW Holley Center for Agriculture and Health, Ithaca, NY, USA; ³Centro Universitário UNIRG, Gurupi, TO, Brasil; ⁴Instituto Federal de Educação, Ciência e Tecnologia de Goiás, Aparecida de Goiânia, GO, Brasil

Correspondence: montalva.cristian@gmail.com

A *Leptolegnia chapmanii*-like oomycete is reported in Brazil for the first time. These aquatic oomycetes were baited with *Aedes aegypti* sentinel larvae in stagnant, transitory breeding sites in secondary tropical gallery forests from September 2014 to February 2015 (spring–summer) in the State of Goiás, Central Brazil. The measurements of major characters of this fungus (given as lower and higher means of two separate series of 25 measurements) are as follows: hyphae 7–7.5 µm wide, zoosporangia 814.1–1297.8 × 11.1–12.2 µm with zoospores formed in a single file, primary zoospore cysts 10.9–11.3 µm in diameter, oogonia 31–32.6 µm in diameter decorated by finger-like projections, and oospores (11.3 µm in diameter, n=25). Overall morphology made it clear that this isolate is closely related to *Leptolegnia chapmanii*. The pathogenicity of 15 isolates was tested under laboratory conditions against *A. aegypti* larvae (L2/L3). Rapid development of disease was observed for five isolates resulting to a complete mortality of tested larvae at a 36 h of exposure to the oomycete. The larvicidal activity of the other isolates was lower, resulting in fewer dead larvae in the same period (< 50%). The findings of this study contribute to a better vision about the geographical distribution of this important entomopathogen and suggest that its function and prevalence as a natural control agent of mosquitoes in South America so far has been probably underestimated.

POSTER Wednesday 16:30 **FU-15**

The puzzle of identifying mosquito-pathogenic isolates of *Leptolegnia*

*Richard A. Humber*¹, *Cristian Montalva*², *Christian Luz*²

¹USDA-ARS Collection of Entomopathogenic Fungal Cultures, RW Holley Center for Agriculture and Health, Ithaca, NY, USA;

²Laboratório de Patologia de Invertebrados, Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, GO, Brasil

Correspondence: richard.humber@ars.usda.gov

The first Brazilian collections of a *Leptolegnia* species (Peronosporomycetes: Saprolegniales) pathogenic for mosquitoes illustrate how difficult it can be to identify some taxa, even in this age of phylogenetic systematics. Apparently, the only existing living cultures of *Leptolegnia chapmanii* Seymour, the sole mosquito-pathogenic species of its genus, in major culture collections were isolated from several sites in the United States and one in Argentina. All of these cultures were isolated after *L. chapmanii* was described. No living authentic or ex-type material of *L. chapmanii* seems to be available for taxonomic comparisons, and the exact location of the holotype specimen of *L. chapmanii* is uncertain. This poster illustrates how the vital information inherent to the descriptions and typification of species names needs to be applied in a real-world setting to a significant biological problem that affects the way in which biodiversity information for Brazil and, indeed, for the entire continent of South America needs to be recorded. And the answer regarding how the Brazilian isolates of *Leptolegnia* should best be identified will also profoundly impact how the scientific world should regard the distribution and comparative frequency of incidences of a taxon such as *Leptolegnia chapmanii*.

POSTER Wednesday 16:30 **FU-16**

A treasure among the trash: *Pandora bullata* from a Brazilian garbage dump

*Richard A. Humber*¹, *Cristian Montalva*², *Karin F.S. Collier*³, *Christian Luz*²

¹USDA-ARS Collection of Entomopathogenic Fungal Cultures, RW Holley Center for Agriculture and Health, Ithaca, NY, USA;

²Laboratório de Patologia de Invertebrados, Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, GO, Brasil; ³Centro Universitário UNIRG, PROPESQ, Gurupí, TO, Brasil

Correspondence: richard.humber@ars.usda.gov

Fungi are where one finds them, and if one seeks fungal pathogens affecting flies, then a garbage dump may be an ideal place to find persistent and abundant fly populations and their fungal pathogens. An obvious fungal epizootic affecting blue bottle flies, *Chrysomya megacephala* (Calliphoridae), was found in the local garbage dump outside of the city of Cavalcante, northern Goiás. The outbreak was observed over three consecutive days in mid-February 2015. This site harbored very large populations of a muscid fly (possibly *Musca domestica*) as well as of *C. megacephala* but only the calliphorids were found to be heavily mycotized by a fungus that can now be unequivocally identified as the first Brazilian (and South American) findings of *Pandora bullata* (Entomophthorales: Entomophthoraceae). Very few infected individuals of *Chrysomya* were found at this site in early April, but those cadavers included small numbers of the characteristically bullate resting spores of *P. bullata*. We do not know whether such abiotic factors as latitude (13°46'40.53" S), day length, or even precipitation patterns in this mid-tropical, montane site might somehow diminish the capacity of this fungus to produce the much more abundant resting spores characteristically seen for entomophthoraceous entomopathogens in more temperate latitudes. During the course of this epizootic 261 individuals were collected in February; of these, 187 (71.6% of the population) were females, 74 (28.4%) were males, but the overall sex ratio within the healthy fly population on this site was not determined.

POSTER Wednesday 16:30 **FU-17-STU**

Effects of temperature on germination, growth, and sporulation of *Culicinympes* species

Juscelino Rodrigues¹, Richard A. Humber², Éverton K. K. Fernandes¹, Christian Luz¹

¹Laboratório de Patologia de Invertebrados, Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, GO, Brazil; ²USDA-ARS Collection of Entomopathogenic Fungal Cultures, RW Holley Center for Agriculture and Health, Ithaca, NY, USA

Correspondence: juscelinor@hotmail.com

Species of *Culicinympes* (Hypocreales) facultatively infect the aquatic larval stages of a range of mosquitoes and blackflies through the digestive tract after conidia ingestion. While *C. clavisporus* was most studied in the 1980s, little is yet known about how temperature affects the development and sporulation of various isolates. Conidia of ten *C. clavisporus* isolates (ARSEF 372, 582, 644, 706, 964, 1260, 2471, 2478, 2479, 2480) and one *C. bisporalis* (ARSEF 1479) were inoculated on SDAY/4 and incubated at 15, 20, 25, 30 or 35°C. Conidial germination was assessed after 24 and 48 h of incubation. Colony sizes were measured daily for 15 days, and conidial production was quantified after 15 days. The best conidial germination rates (>95%) after 48 h of incubation were obtained at 25 and 20°C; the poorest germination (<12%) was at 35°C. The best temperature for radial growth was 25°C (>11.8mm) (followed by 20°C) for all isolates; ARSEF 706, 582 and 372 showed the most vegetative growth (>20mm). There was little growth at 30°C (<2.5mm), and none at 35°C. The maximal conidial production occurred at 25°C for all isolates (≥1.42x10⁷conidia/plate), and the isolates ARSEF 964, 2479, and 644 produced the largest numbers of conidia (≥4x10⁷conidia/plate). After testing these basic growth properties,

the isolates producing the most conidia on the medium surface (964) or on submerged mycelium (644, 2479) were passed three times through *Aedes aegypti* larvae before re-testing the isolates' conidial production and to assess their comparative virulence.

POSTER Wednesday 16:30 **FU-18-STU**

Beauveria bassiana* inhibits host seeking behavior of *Anopheles stephensi

Minehiro Ishii^{1,2,3}, Masanori Koike³, Daigo Aiuchi⁴

¹The United Graduate School of Agricultural Sciences, Iwate University, Japan; ²Research Fellowship for Young Scientists, Japan Society for the Promotion of Science; ³Department of Agro-environmental Science, Obihiro University of Agriculture & Veterinary Medicine, Japan; ⁴Research Center for Global Agro-medicine, Obihiro University of Agriculture & Veterinary Medicine, Japan

Correspondence: aigo@obihiro.ac.jp

In our previous study, various entomopathogenic fungi were isolated from wild adult mosquitoes. Among them, *Beauveria bassiana sensu lato* 60-2 showed highest virulence against *Anopheles stephensi*. We also found new pathway of fungal infection via mosquito proboscis, and fungal development was observed from their head compared with other parts. Female mosquitoes search target hosts using multiple sensory inputs, including CO₂, and non-olfactory stimuli such as heat and visual cues. They have such sensory organs in their head. So the present study was focused on the alteration of the host searching behaviors by entomopathogenic fungus infection. The adult female mosquitoes were inoculated with *B. bassiana s.l.* 60-2, and host searching behavior to heat (40°C) and color (black) were evaluated by using automated-recording device, and host searching behavior to odor (CO₂) was evaluated by using Y-tube olfactometer. Consequently, attractiveness to the heat was drastically decreased from 3 days post inoculation (dpi), and attractiveness to the color had a tendency to decrease from 6 dpi. Furthermore, attractiveness to the odor was decreased from 2 dpi. Our results showed that the mosquitoes could not recognize the heat, visual and odor cues. In other words, they might not be able to recognize hosts by entomopathogenic fungus infection. Conventional vector control method was evaluated only the death of vector. If such kind of behaviors will be inhibited, it also could prevent the disease transmission. So our further study will focus on not only the death of organisms but also "the death as vectors" by entomopathogenic fungi.

POSTER Wednesday 16:30 **FU-19**

Biological activity of entomopathogenic fungi over two species of sugarcane borer *Diatraea* spp. (Lepidoptera: Pyralidae)

Gloria P. Barrera¹, Emiliano Barreto², Paula Sotelo¹, Paola Cuartas¹, Laura Villamizar¹

¹Corporación Colombiana de Investigación Agropecuaria, Corpoica, Mosquera, Colombia; ²Centro de Bioinformática, Instituto de Biotecnología, Universidad Nacional de Colombia, Bogotá, Colombia

Correspondence: pcuartas@corpoica.org.co

Manufacture of the main food product obtained from the sugarcane in Colombia depends of sucrose content, which is seriously affected by the stem borers complex including *Diatraea saccharalis* and *Diatraea indiginella*. Due to the disadvantages of chemical control of these pests, the biological control with entomopathogenic microorganisms is an excellent alternative. In this work, five geographical entomopathogenic fungi were taxonomically classified and biologically evaluated as alternative

for biological control of *D. saccharalis* and *D. indiginella* larvae. The fungi proceeded from different insect species. *Metarhizium* sp. (Mt015) from *Premnotrypes vorax* (Coleoptera: Curculionidae), *Isaria* sp. (Pc013) from *Bemisia tabaci* (Homoptera: Aleyrodidae), *Nomuraea* sp. (Nm006) from *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and *Beauveria* sp. from *P. vorax* (Bb025) and *Diatraea* sp. (Bv062). Molecular identification of fungi was performed using PCR and sequencing of two genomic regions of ribosomal RNA, *ITS* and *18S* genes and *elongation factor-1 alpha* (*EF1- α*). The biological characterization was performed using a bioassay by inoculating of second instar larvae of each specie with fungi suspensions (10^7 conidia/mL). The fungi were classified as *Metarhizium robertsii*, *Isaria javanica*, *Nomuraea rileyi* and the two isolates of *Beauveria* sp. as *B. bassiana*. The efficacies over *D. saccharalis* larvae were 73.3% (Bv062), 63.3% (Mt015), 56.6% (Nm006) and 26.6% (Bb025). The efficacies over *D. indiginella* larvae were 83.3% (Bv062), 80% (Bb025), 66.6% (Mt015) and 26.6% (Pc013). The highest larvae mortalities for these two species of sugarcane borers were obtained with the *B. bassiana* isolated from *Diatraea* spp. (Bv062), showing the adaptation of entomopathogenic fungi to the host from which were isolated.

POSTER/ Wednesday 16:30 **FU-20**

Entomopathogenic fungi for the control of *Thaumastocoris peregrinus* Carpintero and Dellappé (Heteroptera: Thaumastocoridae)

*Sofía Simeto*¹, *Ana B. Corallo*³, *Sandra Lupo*³, *Lina Bettucci*³, *Demian Gómez*², *Paula González*¹, *Gonzalo Martínez*¹, *Eduardo Abreo*², *Federico Rivas*², *Nora Altier*².

¹Instituto Nacional de Investigación Agropecuaria, Programa de Producción Forestal, E.E. del Norte, Tacuarembó; ²Instituto Nacional de Investigación Agropecuaria, Programa de Sustentabilidad Ambiental, Sección Bioinsumos, E.E. Wilson Ferreira Aldunate, Las Brujas, Canelones; ³Facultad de Ciencias/Ingeniería, Laboratorio de Micología, Universidad de la República, Montevideo.

Correspondence: ssimeto@tb.inia.org.uy

The bronze bug, *Thaumastocoris peregrinus* (Heteroptera: Thaumastocoridae) is one of the most important pests of *Eucalyptus* plantations worldwide. Its lacerate-and-flush feeding habit produces foliage blight and defoliation, thus reducing photosynthesis and leading to the death of mature trees when severe infestation occurs. Chemical control is seldom used in commercial forestry because of environmental and economical disadvantages; therefore biological control is seen as the only feasible option. In this work, entomopathogenic fungi were isolated from dead and mycosed bronze bug from *Eucalyptus* plantations in Uruguay. Fungi identification was based on spore and sporulating structures morphology, culture characteristics, and sequence analysis of the ribosomal DNA comprising *ITS1*, *ITS2* and the *5.8S* subunit. The identified entomopathogenic fungi belonged to the genera *Beauveria*, *Isaria*, *Lecanicillium*, *Purpureocillium* and *Pochonia*. Fifty-eight entomopathogenic fungal isolates from bronze bug and other forestry and agricultural pest insects were tested for their pathogenicity and virulence against *T. peregrinus*. A first *in vitro* screening was made by spraying 10^7 spores/ml conidial suspensions onto adults of *T. peregrinus* reared in mesh cages on detached leaf-bearing twigs of *E. tereticornis*. Strains were classified into four categories based on the number of days to reach 90% of mortality. Most of the tested strains (80%) were pathogenic to bronze bug, and showed different degrees of virulence. Values of LC_{50} and L_{50} , spore germination rate at different temperatures, water activity and light exposure are being determined on a second round of

selection. The resulting most promising strains will be subjected to mass production studies.

POSTER Wednesday 16:30 **FU-21**

Effectiveness of *Metarhizium anisopliae* and *Beauveria bassiana* against eggs of tomato leafminer, *Tuta absoluta* Meyrick, 1917 (Gelichiidae: Lepidoptera)

*Laila A. M. Al-Shuraym*¹, *Nagdy F. Abdel-Baky*²

¹Department of Biology, College of Arts and Sciences in Buraydah, Qassim University, Saudi Arabia; ²Department of Plant Production & Protection, College of Agriculture and Veterinary Medicine Qassim University, P.O. Box: 6622, Buraydah 51452, Saudi Arabia
Correspondence: nafabdel2005@yahoo.com

The tomato leaf-miner, *Tuta absoluta* Meyrick is a very serious and dangerous leaf mining, stalk borers and fruits pest of Solanaceae plants (eggplants, potatoes, sweet peppers, other various cultivated plants and certain weeds of Solanaceae family), but tomatoes are considered the most preferred host plants. This insect pest has an ability to cause great reduction in tomato fruits ranged from 50-100% of the yield and so, the insect presence may leads to limit the tomato exports among countries and to several destinations. So, this study was carried out to assay the effectiveness of two entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* against *T. absoluta* eggs under lab conditions. As well as, the planning use of these entomopathogenic fungi as bio-insecticides to reduce the insecticide use within IPM programs was discussed. In this paper, the ability of *M. anisopliae* and *B. bassiana* in reducing the vitality of *T. absoluta* eggs was assessed. Eggs of *T. absoluta* were treated with different conidial concentrations of the two entomopathogenic fungi under laboratory conditions. The obtained results suggest that the moderate (8×10^7) and the higher (10×10^7) spore concentration/ml of *M. anisopliae* and *B. bassiana* were the effective conidial concentrations against *T. absoluta* eggs and could be developed into bio-control agents against *T. absoluta* eggs in IPM programs with another safety control tools.

POSTER Wednesday 16:30 **FU-22**

***Metarhizium* F52 microsclerotia applied in hydromulch to control Asian longhorn beetle**

*Tarryn Anne Goble*¹, *Ann Hajek*², *Mark Jackson*², *Sana Gardescu*¹

¹Department of Entomology, Cornell University, Ithaca, NY, 14853-2601; ²USDA-ARS-NCAUR, Crop Bioprotection Research Unit, 1815 N University Street, Peoria, IL, 61604

Correspondence: tazgoble@gmail.com

Metarhizium brunneum F52 (Hypocreales: Clavicipitaceae) produces environmentally persistent microsclerotia, which can be mixed with a carrier (clay, carboxymethyl-cellulose, or diatomaceous earth) to produce granules. These are incorporated into hydromulch (water, wheat straw mulch and psyllium tackifier), which holds moisture and allows microsclerotia to produce many conidia. The formulation represents a novel mycoinsecticide to spray onto trunks and branches of trees. No significant differences were observed in conidia production or viability between granule types, for five water activities (α_w), averaging 1.74×10^9 conidia/g at the highest α_w (0.983) compared to 3.45×10^6 conidia/g at the lowest α_w (0.929). F52-hydromulch reduces Asian longhorned beetle, *Anoplophora glabripennis* fecundity. Breeding pairs exposed to logs sprayed with F52-hydromulch had only 3.9 viable offspring per female, versus 18.3 for controls. When beetles were exposed by walking 80 cm on a log sprayed with microsclerotia in hydromulch, median time survived was 18-22 days, but 23-26 days using logs sprayed with

microsclerotia only, which produced fewer conidia. Conidial production and field persistence were evaluated using substrates sprayed with hydromulch and exposed on forest tree trunks. Conidial densities increased over time, reaching 5.5×10^6 conidia/cm² by 20-30 d during periods of higher temperature, humidity and rainfall. Increased conidial densities resulted in significantly decreased survival of *A. glabripennis*, especially females, in laboratory bioassays using field-collected sprayed substrate. Females exposed 2 d to high conidial densities had median survival times of 16-22 d. The results suggest reapplication might only need to occur after 6-8 weeks.

POSTER Wednesday 16:30 **FU-23-STU**

Control of ticks (*Ixodes ricinus*) in sheep pastures in Norway with the fungal biocontrol agent BIPESCO 5 (*Metarhizium brunneum*)

*Natasha Iwanicki*¹, *Lise Grøva*², *Hermann Strasser*³, *Annette Folkedal Schjøll*², *Maria Björkman*², *Karin Westrum*², *Jürg Enkerli*⁴, *Nicolai V. Meyling*⁵, *Ingeborg Klingen*²

¹Department of Entomology and Acarology, ESALQ, University of São Paulo, Brazil; ²Norwegian Institute for Agricultural and Environmental Research (Bioforsk), Norway; ³Institute of Microbiology, Leopold-Franzens University of Innsbruck, Austria; ⁴Swiss Federal Research Station for Agroecology and Agriculture, Switzerland; ⁵Department of Ecology, Faculty of Life Sciences, University of Copenhagen

Correspondence: ingeborg.klingen@bioforsk.no

Tick-borne fever, vectored by the tick *Ixodes ricinus*, is a major challenge in Norwegian sheep farming during the grazing season. Field trials were carried out to evaluate the efficacy of the entomopathogenic fungus *Metarhizium brunneum*, BIPESCO 5, in controlling natural tick populations in two sheep pastures. In 2013, plots were treated with BIPESCO 5 as: no fungus (control), fungus application in May or fungus application in May and September. In 2014, another pasture was treated either with no fungus (control), fungus application in May and June, or fungus application in May, June and September. Tick populations were monitored by flagging and incubated for fungal infection. The application of *M. brunneum* BIPESCO5 reduced the tick population in fungus treated plots but <15% of ticks with *Metarhizium* spp. were observed. Baiting of soil samples prior to fungus application with wax moth larvae *Galleria mellonella* showed very low natural occurrence of *Metarhizium* spp. at the first field site. The retrieved fungal isolates from ticks are being molecularly characterized by microsatellite markers to verify if infections were caused by BIPESCO 5 or naturally occurring fungi.

POSTER Wednesday 16:30 **FU-24-STU**

Development of *Aphidius colemani* incapable of oviposition and the evaluation of control efficacy against cotton aphid by combined use of entomopathogenic fungus and parasitoid wasp

*Fuka Oikawa*¹, *Minehiro Ishii*^{2,3}, *Masanori Koike*¹, *Daigo Aiuchi*⁴
¹Department of Agro-environmental Science, Obihiro University of Agriculture and Veterinary Medicine, Japan; ²The United Graduate School of Agricultural Sciences, Iwate University, Japan; ³Research Fellowship for Young Scientists, Japan Society for the Promotion of Science; ⁴Research Center for Global Agro-medicine, Obihiro University of Agriculture and Veterinary Medicine, Japan

Address for Correspondence: aigo@obihiro.ac.jp

In our previous study, entomovectoring ability of *Aphidius colemani* to carry *Lecanicillium muscarium* was reported. However, it was not possible to evaluate the single control efficacy of insect-vectored fungus against cotton aphid, so we could not judge whether this efficacy is synergistic or additive.

The present study aimed to develop *A. colemani* incapable of oviposition, and evaluation of control efficacy against cotton aphid by combined use of entomopathogenic fungus and parasitoid wasp were conducted. The tip of the ovipositor of parasitoid was closed by adhesive, and then the abilities of *A. colemani* to host searching and/or oviposition behavior were evaluated. Consequently, there were no significant difference between parasitoid incapable of oviposition and parasitoid capable of oviposition on the number of host searching and ovipositional behavior. This result suggests that both parasitoids were equivalently contact with the aphid host and carrying entomopathogenic fungus to aphid population equally. So this parasitoid was applied for bioassay as vector of *L. muscarium* without oviposition, namely fungus single inoculation plot. The evaluation of control efficacy against cotton aphid by combined use of parasitoid and entomopathogenic fungi was resulted in no significant difference between *L. muscarium* single inoculation plot and combined use plot on the number of fungus infected aphid. Moreover, there were no significant difference between *A. colemani* released plot and combined use plot on the number of mummy. From the above results, it was revealed that the cotton aphid control by combined use of *A. colemani* and *L. muscarium* will operate "additional" effect.

POSTER Wednesday 16:30 **FU-25**

Latent infection of wireworms and its relation to ambient *Metarhizium* levels in soil

Todd Kabaluk, *Erica Li-Leger*

Agriculture and Agri-Food Canada, Agassiz, BC, Canada

Correspondence: Todd.Kabaluk@agr.gc.ca

Wireworms (Coleoptera: Elateridae) are serious agricultural pests, with soil-dwelling larvae attacking roots and tubers of crop plants, and fruit in contact with the soil surface. Researchers collect wireworms for laboratory experiments to study of their behaviour and test pest control agents but frequently lose them to *Metarhizium* Petch (Ascomycota: Hypocreales: Clavicipitaceae) infection in the absence of an inundative application. We found *Metarhizium* infection in 13-100% of live wireworms acquired directly from field collections, and in wireworms collected in the field and maintained indoors. *Metarhizium* DNA in living wireworms maintained indoors sometimes exceeded 250 pg/ug total DNA (0.025% of wireworm mass). Expressed as copies of *Metarhizium* DNA/g, soil levels of *Metarhizium* ranged from 4,037 in agricultural field soil to 721,538 (soil harbouring a wireworm collection indoors). The quantity of soil *Metarhizium* was related to the incidence of infection of live specimens as % infected wireworms = 34.03 log[copies Met DNA/g soil] - 99.90; R² = 0.98. These findings have implications for *Metarhizium* as a regulator of wireworm populations in nature, as a biological control agent for wireworms, storage of specimens, and raise questions about wireworm immune response.

POSTER Wednesday 16:30 **FU-26**

Smell the danger! Odor-perception of fungal infection risk in a below-ground parasitoid

*Belén Cotes*¹, *Linda-Marie Rännbäck*¹, *Maria Björkman*², *Hans R Norli*², *Nicolai V Meyling*³, *Birgitta Rämert*¹, *Peter Anderson*¹

¹Department of Plant Protection Biology, Division of Integrated Plant Protection, Swedish University of Agricultural Sciences, Växtskyddsvägen 3, P.O. Box 102, SE-230 53 Alnarp, Sweden;

²Bioforsk. Plant Health and Plant Protection Division, Høgskoleveien 7, 1430, Ås, Norway; ³Department of Plant and Environmental Sciences, University of Copenhagen, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark

Correspondence: nvm@plen.ku.dk

To locate and evaluate host patches before oviposition, parasitoids of herbivorous insects utilize plant volatiles and host-derived cues, but also evaluate predator-derived infochemicals to reduce predation risks. When foraging in host habitats infested with entomopathogenic fungi, parasitoids may reduce the risk of fungal infection by avoiding such patches. In this study, we examined whether the presence of the entomopathogenic fungi *Metarhizium brunneum* and *Beauveria bassiana* in soil habitats of a root herbivore, *Delia radicum*, affects the behavior of *Trybliographa rapae*, a parasitoid of *D. radicum*. Olfactometer bioassays revealed that *T. rapae* avoided fungal infested host habitats and that this was dependent on fungal species and density. In particular, the parasitoid avoided habitats with high densities of the more virulent fungus, *M. brunneum*. In contrast, host density was found to be important for attraction of *T. rapae*. Volatiles collected from host habitats revealed different compound profiles depending on fungal presence and density, which could explain the behavior of *T. rapae*. We conclude that *T. rapae* females may use volatile compounds to locate high densities of prey, but also compounds related to fungal presence to reduce fungal infection risk towards themselves and their offspring.

POSTER Wednesday 16:30 **FU-27-STU**

Maize endophytes offer potential protection against insects and disease

Jenny J. Brookes, Travis R. Glare, Michael Rostals

Bio Protection Research Centre, Lincoln University, New Zealand

Correspondence: jenny.brookes@lincoln.ac.nz

Endophytes of plants are increasingly been seen as including species beneficial to the growth of plants. In some cases the presence of endophytes results in increased tolerance to insect pests or plant diseases. In this study, endophytes have been isolated from maize plants over two seasons and identified, to determine the full complement of fungi associated with maize in New Zealand. Endophytic ability is being confirmed by re-establishment in maize plants. Bioassays using several insect species and a disease of maize are being used to identify beneficial endophytes. It is hoped that this work will expand the range of species known to improve maize growth.

POSTER Wednesday 16:30 **FU-28**

Physiochemical characteristics of entomopathogenic fungi-derived antifungal substances

Tae Young Shin, Sung Min Bae, Won Seok Gwak, Seung Hee Lee,
Yong Oh Ahn, Soo Dong Woo

Department of Agricultural Biology, College of Agriculture, Life & Environment Science, Chungbuk National University, Cheongju 361-763, Korea

Correspondence: sdwoo@cbnu.ac.kr

The bioactive substances from entomopathogenic fungi have been suggested as potential candidates for the development of new bioactive agents. Our previous study suggested that a lot of entomopathogenic fungi have antifungal activity against plant pathogenic fungus *Botrytis cinerea* on solid medium. Additionally, their culture filtrates suppressed the growth of *B. cinerea*, indicating that the suppression was due to the presence of antifungal substances in the fungal culture filtrates. In this study, therefore, the physiochemical characteristics, such as thermal stability, polarity, proteases susceptibility and molecular weight, of the antifungal substances from fungal culture filtrates were investigated to evaluate their usefulness. As the results, the

antifungal substances from various fungi had various features according to the isolated fungus. Most of all antifungal substances were hydrophilic and stable at high temperature (100°C, 15 min). They were also resistant to degradation by protease and had various range of molecular weights. These antifungal substances may be a good candidates to be used in the development of a new biocontrol method.

POSTER Wednesday 16:30 **FU-29-STU**

Entomopathogenic fungi "infecting" young lives

Alap Sahoo¹, Sumanth S. R. Dara¹, Suchitra S. Dara², Surendra K. Dara³

¹Massachusetts Institute of Technology, Cambridge, MA, USA;

²Warren Junior High School, Bakersfield, CA, USA; ³University of California Cooperative Extension, San Luis Obispo, CA, USA.

Correspondence: skdara@ucdavis.edu

Working with entomopathogenic fungi has changed our lives and academic careers. We went from people who were reluctant to even look at insects, let alone handle them, to people who could carry out major scientific research projects. Engaging in real life research at a young age helped us know about the specialized area of entomopathology and develop research skills that are very important in life. Among other skills, we learned how to plan and execute scientific studies, prepare for challenges, adapt to changing situations, learn to be patient, engage in the meticulous process of data collection, do proper data analysis, and write and present results. After all of this work, it is truly rewarding because we were able to contribute to science and provide practical solutions. Various laboratory experiments we conducted with *Beauveria bassiana* led to field studies against major arthropod pests such as *Lygus hesperus*, *Bagrada hilaris*, *Tetranychus urticae* in strawberries and vegetables.

Microbial Control

POSTER Wednesday 16:30 **MC-1**

Next generation biopesticides for New Zealand's key insect pests and plant diseases

Maureen O'Callaghan¹, Travis Glare², Mark Hurst¹, Sean Marshall¹, Tracey Nelson¹, Michael Wilson³, Sarah Mansfield¹, Sue Zydenbos¹

¹Innovative Farm systems, AgResearch, Lincoln Research Centre, New Zealand; ²Bio-Protection Research Centre, Lincoln University New Zealand; ³Innovative Farm systems, AgResearch, Ruakura New Zealand

Correspondence: maureen.ocallaghan@agresearch.co.nz

New Zealand's agricultural and horticultural producers are seeking to reduce their environmental footprint but are vulnerable to major production losses caused by insect pests and diseases. Research is underway to provide farmers and growers with New Zealand-appropriate, cost effective biopesticides for control of intractable indigenous insect species emerging as pests as a result of land use change, farming intensification, and climate change. Targets include key pests complexes such as foliar-feeding pests of brassicas and subtterranean turf pests. Biopesticides are also being developed for control of incursions of exotic cosmopolitan pests and diseases such as diamondback moth, African black beetle. To minimise regulatory hurdles, microorganisms under development as biopesticides include new species/strains isolated from productive ecosystems within New Zealand, such as the broad host range insecticidal bacterium

Yersinia entomophaga, *Brevibacillus* sp.; and fungal species (*Metarhizium* and *Beauveria*) are being developed for independent and dual use (synergy) for the control of insect pests. A range of formulations and delivery systems (e.g. baits, sprays, granules, seed treatments) are being developed. Prototype biopesticides are being trialled in field tests, in conjunction with industry partners. For biopesticides to succeed in mainstream agriculture and horticulture in New Zealand, new biopesticide products will need to have levels of efficacy comparable to currently available controls, and be able to be used by farmers with minimal change of practice.

POSTER Wednesday 16:30 **MC-2**

Appearance of pathogens within outbreak populations of native porina caterpillar (*Wiseana* spp.) populations in New Zealand

Sean D. G. Marshall¹, Richard J. Townsend², Colin M. Ferguson²,
Sarah Mansfield¹

¹Innovative Farming Systems, AgResearch, Lincoln Research Centre, Christchurch, New Zealand; ²Innovative Farming Systems, AgResearch, Invermay Agricultural Centre, Mosgiel, New Zealand
Correspondence: sean.marshall@agresearch.co.nz

The Gondwanaland remnant of Aotearoa-New Zealand developed a unique flora and fauna over >60 million years. When settlers introduced European grasses and clovers they initially grew spectacularly in the absence of their usual pest complex. However, some native herbaceous insects were capable of adapting to the new resources and reached unprecedented numbers destroying the developing pastures until the associations between native herbivorous insects and population regulating systems could be re-established. Today, an uneasy balance of nature has been reached across many New Zealand grasslands, except where major new land conversions have caused fresh pest outbreaks. The new large scale pasture development that is happening on the New Zealand West Coast ("flipping" of swamps to create new pastures), central North Island Volcanic Plateau (conversion of pine forest to dairy pasture), and in Otago (development of largely undisturbed soil) has reset the conditions to total environmental disruption. After initial vigorous pasture growth, the endemic porina caterpillar (*Wiseana* spp.), normally only present at >20/m², reaches numbers exceeding 800/m² causing extensive damage within 1-3 years of land development. The enemy release hypothesis states that invasive species thrive in new environments due to the lack of natural enemies present in their home range. While this hypothesis has generally been investigated for exotic invasions around the world, invading organisms can also be indigenous, moving into new or disrupted ecosystems. Research has indicated a growing awareness of the importance of pathogens in population regulation. We have discovered disease is beginning to infect porina caterpillar populations found in recently modified New Zealand lands and will discuss our current findings.

POSTER Wednesday 16:30 **MC-3**

Pathogens and nematodes of bark beetles (Coleoptera: Scolytidae) in Georgia coniferous forest

Archil Supatashvili, Medea Burjanadze

Department of Forest Protection, Vasil Gulisashvili Forest Institute, Agricultural University of Georgia, Tbilisi, Georgia

Correspondence: m.burjanadze@agruuni.edu.ge

Georgia is distinguished by its biodiversity, with endemic and relict species of plants in Caucasus. *Picea orientalis*, *Pinus sosnovskiy* and *Pinus Eldaric*, are widespread and present in Georgian coniferous forest. Principal species of bark beetles:

Spruce bark beetle - *Ips typographus* L, Great spruce bark beetle - *Dendroctonus micans* (Kugelann), Six-toothed bark beetle - *Ips sexdentatus* (Börner), Pine shoot beetle- *Tomicus piniperda* L. are causing tree mortality throughout the coniferous forest. 50-70-ies of the last century had emerged in forests of Borjomi gorge an extremely harmful insect pest *D.micans*, which caused the death of millions of spruce trees. From 2000, *I. typographus* became a major outbreak in the last decade. Human activity, changing climate, changing physiological conditions of host plants, influence on insect activity, increase it responsibility and risk for new colonization, not only in Georgia, but in Caucasian and Black sea region.

During 2013-2014, our research focused on the control of bark beetles using mechanical methods and pheromone traps. Also we investigated natural enemies: predators and pathogens (microorganisms and parasitic nematodes) which are responsible to make balance density of bark species.

Various pathogen species and nematodes were observed in the populations of bark beetles. *Gregarina typographi*, was found in *I. typographus* (40.5-45.5%) and *I. sexdentatus* (15.3%). *Chytridiopsis typographi* was found in *I. typographus* and *T.piniiperda* (6.3% -12%) and *I. sexdentatus*, (4.5%). Fungus *Beauveria bassiana* (3.5%) was found in *I.typographus*. Nematodes (42.7-65.0%) *Contortylenchus diplogaster*. were observed in *I. typographus*. *Contortylenchus* sp. was found in *I.sexdentatus*, 26.1% nematodes were associated with *D.micans*.

POSTER Wednesday 16:30 **MC-4-STU**

PhopGV for control of *Tuta absoluta* in tomato and *Phthorimaea operculella* and *Tecia solanivora* in potato

Andreas Larem, Eva Fritsch, Karin Undorf-Spahn, Johannes A. Jehle
Julius Kühn-Institut (JKI), Federal Research Centre for Cultivated Plants, Institute for Biological Control, Heinrichstr. 243, 64287

Darmstadt, Germany

Correspondence: andreas.larem@jki.bund.de

Due to increasing standards in food production alternatives are needed to reduce the usage of chemical plant protection agents. A promising method to protect plants against insect caterpillars is the usage of baculoviruses. Previous studies have shown that there may be the opportunity to use a single baculovirus isolate to control three different but close related insect species i.e. *Phthorimaea operculella* (potato tuber moth), *Tecia solanivora* (Guatemalan potato moth) and *Tuta absoluta* (tomato leaf miner). Isolates of *Phthorimaea operculella* granulovirus (PhopGV) were found to infect all of these three pests. To find a highly virulent isolate to control these three pests different PhopGV isolates are characterized by biological and molecular means. To distinguish between different isolates variable regions like ORF 129 which is coding for egt (ecdysteroid UDP-glucosyltransferase) can be a useful tool in combination with restriction fragment length polymorphism (RFLP) analysis. Bioassays give a hint of the host interaction and virulence of the several isolates. As an outcome of this research the development of a combined control of different pests by highly selective baculoviruses is aimed.

POSTER Wednesday 16:30 **MC-5-STU**

Sublethal effects of Cry1Ac on immune responses and baculovirus infection in *Spodoptera frugiperda*

Lucas N. Wisch^{1,2,4}, Jenny S. Cory², Daniel R. Sosa-Gómez³

¹Pos-Graduation Program, State University of Ponta Grossa, Paraná, Brazil; ²Dept. of Biological Sciences, Simon Fraser University, Burnaby, , Canada; ³Entomology Research, Embrapa Soybean, Londrina, Paraná, Brazil; ⁴Supported by Capes

Correspondence: wisch022@hotmail.com

Bacillus thuringiensis (Bt) is widely used in agricultural systems as an alternative strategy for pest management through application as a biopesticide or in genetically modified plants that express Cry proteins. Bt crops can express one or a combination of genes expressing proteins with different activity spectra, such as Bt Soybean (expressing Cry1Ac) designed to control a complex of noctuid larvae. However, the rapid adoption and expansion of the area planted with this crop could be a concern, as the quantity of Cry1Ac expressed is not enough to kill some soybean pests, particularly *Spodoptera* spp. This frequent exposure to low concentrations of Cry1Ac could result in adaptive immune responses and transgenerational effects, such as via immune priming, as well as alter the interaction with other entomopathogens that could simultaneously occur in presence of this microbial agent. We investigated the sublethal effects of the HD-73 strain, that expresses Cry1Ac, on (i) the immune response of *S. frugiperda* larvae, and (ii) virulence and productivity of subsequent baculovirus infection. We will present data on whether the immune response (hemocyte density, phenoloxidase, protein and anti-bacterial activity), and life history parameters (longevity, fecundity and survival) are altered within and between generations in *S. frugiperda* after exposure to Cry1Ac. In addition, we will report the impact of different sublethal doses of Cry1Ac on subsequent baculovirus infections through measures of mortality, speed of kill and yield of occlusion bodies. Sublethal effects of Bt could influence the spread and infection rates of other entomopathogens and thus the efficacy of microbial control agents.

POSTER Wednesday 16:30 **MC-6-STU**

Virulence of a newly isolated *Bacillus thuringiensis* strain against lepidopteran larvae

Anna I. Moldovan^{1,2}, *Natalia Munteanu Molotievskiy*², *Svetlana G. Bacal*², *Ion K. Toderas*²

¹Faculty of Biology and Soil Science, Moldova State University, Chisinau, Republic of Moldova; ²Institute of Zoology, Academy of Sciences of Moldova, Chisinau, Republic of Moldova

Correspondence: anna.moldovan@yahoo.com

A large number of fruit trees, including apples, pears and quinces are cultivated in temperate climates. A rich diversity of insects attacks the fruit trees, among them, lepidopterans being considered the most dangerous pests in many countries in Europe including Moldova, causing substantial yield losses.

We evaluated the virulence of newly isolated *Bacillus thuringiensis* strain CNMN BB-04 to three lepidopteran species, *Cydia pomonella* (L. 1758), *Archips rosana* L. 1758 and *Lymantria dispar* L. 1758, larvae. In bioassay test, apple leaves were used as diet. Leaves of approximately the same size were treated with prepared bacterial suspension, dried at 20°C and placed into petri dishes. Sterile 0.9% NaCl solution was used as control. At least 10 larvae and 2-5 treated leaves were used per repetition. Three repetitions were made for each lepidopteran species. Insect mortality was recorded after 3 days of incubation at 21°C and photoperiod of 24 h and all dead insects were removed from petri dishes. In assays with treated apple leaves, at a concentration of 0.9×10^9 spores/ml, larval mortality of *Archips rosana*, *Cydia pomonella* and *Lymantria dispar* was 90.0, 93.33 and 96.67% respectively. The virulence of the strain was expressed in LC₅₀ values calculated according to Kerber's formula, the highest value 1.392×10^5 spores/ml being recorded for *Lymantria dispar*.

These results show that *Bacillus thuringiensis* strain CNMN BB-04 is virulent to all tested lepidopteran species and has potential as a biological control agent in fruit trees pest management programs.

POSTER Wednesday 16:30 **MC-7**

Selection and characterization of formulation prototypes based of *Beauveria bassiana* for biological control of *Ceratomyxa tingomariana*

*Erika Grijalba*¹, *Lorena Garcia*¹, *Carlos Espinel*¹, *Judith Guevara*², *Hugo Fernando Rivera*¹

¹Biological Control Laboratory; ²Entomological Laboratory, Colombian Corporation for Agriculture Research, CORPOICA, Colombia

Correspondence: egrijalba@corpoica.org.co

A microbial formulation is composed of an active ingredient with biological activity and excipients that are raw materials inert that protect and release the active ingredient. The formulation should ensure stability during production, processing and storage; facilitate application, provide protection against ultraviolet radiation and the temperature. In the biological control laboratory were development nine formulation prototypes corresponding to a granulated and nine formulation prototypes corresponding to an emulsifiable concentrate based of *Beauveria bassiana* (Bv060), isolation previously selected for its efficacy against *Ceratomyxa tingomariana*, pest of soybean crop that reduces the nitrogen fixation and the yield crop. The aim of this work was to select two formulation prototypes based of *B. bassiana* against *C. tingomariana*. Initially, the selection of four prototypes was doing from test of resistance to temperature and to ultraviolet radiation. The prototypes were exposed to a storage temperature of 25°C and 35°C and the germination and Colony Formate Unit (CFU) were evaluated in the zero time and after of 1, 2 and 3 months. Later, the prototypes were reconstituted in Tween 80 0,1% and were exposed to ultraviolet radiation type B (UVB) for different times. Again, was evaluated the germination and Colony Formate Unit (CFU). From these results were selected four prototypes. The prototypes selected were biological testing on laboratory and were determined the lethal concentration 90 (LC90). With the value CL90 be evaluated the efficacy of the prototypes in greenhouse.

POSTER Wednesday 16:30 **MC-8**

Sublethal effects of Cry1Ac *Bacillus thuringiensis* protein on *Plutella xylostella* (Lepidoptera: Plutellidae) Brazilian population: Life table study

*Caroline P. De Bortoli*¹, *Ricardo A. Polanczyk*¹, *Neil Crickmore*², *Sergio A. De Bortoli*¹, *Alessandra M. Vacari*¹

¹Department of Plant Protection, Sao Paulo State University, Jaboticabal, Sao Paulo, Brazil; ²Department of Biochemistry, School of Life Sciences, University of Sussex, Falmer, Brighton, United Kingdom

Correspondence: carubortoli@yahoo.com.br

The sublethal effects of Cry1Ac *Bacillus thuringiensis* protein were investigated on life table parameters of the *Plutella xylostella* (L.) Brazilian population. After dip-leaf bioassay with kale leaves, the sublethal concentrations 0.5, 0.25, and 0.1 µg/mL were determined. The control group was water and Tween 0.05%. Third instar *P. xylostella* larvae were placed on kale leaves after dry and the evaluations were performed in 3 days. The results showed that the mean values of the life table parameters were significantly affected by treatment. The net reproductive rate (R_0), intrinsic rate of increase (r_m), and finite rate of increase (λ) decrease with the increase of concentrations of Cry1Ac protein. The values of R_0 were 64.6 females/female to control, 28.8 females/female to 0.1 µg/mL, 21.1 females/female to 0.25 µg/mL, and 20.8 females/female to 0.5 µg/mL. The values of r_m were 0.303 females/female*day to control, 0.218 females/female*day

to 0.1 µg/mL, 0.206 females/female*day to 0.25 µg/mL, and 0.195 females/female*day to 0.5 µg/mL. The mean generation time (T) and doubling time (Dt) increase with the increase of concentrations of Cry1Ac. The values of T were 15.6 days to 0.5 µg/mL, 15.4 days to 0.5 µg/mL, 14.9 days to 0.25 µg/mL, and 13.7 days to control. The values of Dt were 3.5 days to 0.5 µg/mL, 3.3 days to 0.5 µg/mL, 3.2 days to 0.5 µg/mL, and 2.3 days to control. The sublethal concentrations of Cry1Ac protein may reduce the population of *P. xylostella* by decreasing its survival and reproduction, and by delaying its development.

POSTER Wednesday 16:30 **MC-9**

Interaction between predator *Podisus nigrispinus* (Dallas) and the entomopathogenic bacterium *Bacillus thuringiensis* Berliner
Vanessa F. P. Carvalho, Sergio Antonio De Bortoli, Alessandra M. Vacari

Department of Plant Protection, Sao Paulo State University, Jaboticabal, Sao Paulo, Brazil

Correspondence: bortoli@fcav.unesp.br

The use of the insecticide the basis of *Bacillus thuringiensis* Berliner in combination with other management tactics can be good strategy for overcoming the resistance. The aim of this research was to verify the effect of *B. thuringiensis* on the predator *Podisus nigrispinus* (Dallas), providing suspension of the bacteria HD1 strain or commercial product as water source. The predators fed regularly with *Diatraea saccharalis* (F.) larvae as prey. Three treatments were performed: *B. thuringiensis* kurstaki HD1 strain (3×10^8 spores/mL), commercial product suspension (*B. thuringiensis* aizawai - Agree[®]), and water (control). The commercial product was used in the concentration recommended by the manufacturer. We evaluated the survival, number of eggs per female and sex ratio of the offspring. This information was used to estimate the fertility life table parameters. The survival to adulthood was 60.0% for the control treatment, 44.0% for HD1 strain, and 40.0% for the Agree[®] product. The female fecundity was 200.8 eggs/female for the control treatment, 112.0 eggs/female for the HD1 strain, and 106.9 eggs/female for the Agree[®] product. The population parameters of fertility life table R_0 , r_m , T, and DT were 62.9 females/female, 0.142 females/female*day, 29.2 days, and 4.9 days for the control treatment; 36.9 females/female, 0.137 females/female*day, 26.4 days, and 5.0 days for the strain HD1; 31.5 females/female, 0.135 females/female*day, 25.5 days, and 5.1 days for the Agree[®], respectively. The HD1 strain and the Agree[®] product affect the biological characteristics and population parameters of *P. nigrispinus* when the predators ingest suspension.

POSTER Wednesday 16:30 **MC-10**

Reproduction and population parameters of predator *Podisus nigrispinus* (Dallas) providing *Bacillus thuringiensis* Berliner suspension as water source over generations

Vanessa F. P. Carvalho, Sergio Antonio De Bortoli, Alessandra M. Vacari

Department of Plant Protection, Sao Paulo State University, Jaboticabal, Sao Paulo, Brazil

Correspondence: amvacari@gmail.com

A better control strategy could be to use a combination of *Bacillus thuringiensis*-based insecticide along with other management tactics, such as the use of other natural enemies, to try to maintain the balance in the agroecosystem. The aim of this research was to verify the effect of *B. thuringiensis* on the predator *Podisus nigrispinus* (Dallas), providing suspension of HD1 strain or commercial product as water source over generations.

The predators fed regularly with *Diatraea saccharalis* (F.) larvae as prey. Three treatments were performed: *B. thuringiensis* kurstaki HD1 strain (3×10^8 spores/mL), commercial product suspension (*B. thuringiensis* aizawai - Agree[®]), and water (control). The commercial product was used in the concentration recommended by the manufacturer. The third generation of predators submitted of treatments was evaluated. We evaluated the survival, number of eggs per female and sex ratio of the offspring. This information was used to estimate the fertility life table parameters as net reproduction rate (R_0), intrinsic rate of increase (r_m), average generation time (T), and doubling time (Dt). The population parameters of fertility life table R_0 , r_m , T, and Dt were 88.8 females/female, 0.168 females/female*day, 26.7 days, and 4.1 days for the control treatment; 30.4 females/female, 0.121 females/female*day, 28.2 days, and 5.7 days for the strain HD1; 90.4 females/female, 0.177 females/female*day, 25.6 days, and 3.9 days for the Agree[®], respectively. Only HD1 strain affected the population parameters of *P. nigrispinus* when the predators ingest suspension over the generations by reducing the number of females produced per female.

POSTER Wednesday 16:30 **MC-11-STU**

Expression of recombinant ABCC2 from *Pectinophora gossypiella* and their influences on the cytotoxicity of activated Cry1Ac to Hi5 cells

Yutao Xiao

The State Key Laboratory for Biology of Plant Disease and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, West Yuanmingyuan Road, Beijing, 100193, China

Correspondence: xiao20020757@163.com

Bacillus thuringiensis Cry-toxins are widely used for insect control and at the same time development of insect resistance to these toxins is an emerging problem with increasing proportions during recent years. Insect midgut-proteins have been identified as binding sites or receptors for Cry toxins playing important roles in toxicity. Recently, mutations in the ABCC2 transporter were reported to be link to resistance to Cry1Ac in several insect species. Here, we fused ABCC2 from *Pectinophora gossypiella* with GFP and transiently expressed in Hi5 cells by transfection, and GFP-expressing Hi5 cells were used as control. Western blot assays showed that the molecular mass of the expressed PgABCC2-GFP protein was about 170 kDa, which was consistent with the predicted one. PgABCC2-GFP was localized in both cytoplasm and cell membrane, while GFP expressed only was localized in cell nucleus. The efficiency of transfection was more than 50%. Activated Cry1Ac was not toxic to GFP-expressing Hi5 cells at the concentration of 40 µg/ml, but was toxic to PgABCC2-expressing Hi5 cells at the concentration of 0.5 µg/ml. These findings implied that PgABCC2 is an important Bt receptor.

POSTER Wednesday 16:30 **MC-12**

Evaluation of commercial formulations of entomopathogenic fungi for managing the ambrosia beetles *Xylosandrus crassiusculus* and *Xyleborus volvulus* (Coleoptera: Curculionidae), vectors of the laurel wilt pathogen affecting avocado production in Florida

Pasco B. Avery¹, Verónica F. Bojorque^{1,2}, José M. Pérez-Martínez³, Armando Monterroso³, Ronald D. Cave¹, J. Daniel Carrillo⁴

¹University of Florida, IFAS, Indian River Research and Education Center, Fort Pierce, FL, USA; ²PanAmerican School of Agricultural, El Zamorano, Honduras; ³Brooks Tropicals, LLC, Homestead, FL, USA; ⁴University of Florida, IFAS, Tropical Research and Education Center, Homestead, FL, USA

Correspondence: pbavery@ufl.edu

The ambrosia beetles *Xylosandrus crassiusculus* (Xc) and *Xyleborus valvulus* (Xv) (Coleoptera: Curculionidae: Scolytinae) vector the fungal pathogen *Raffaella lauricola*, which causes laurel wilt, a lethal disease of avocado. The objectives of these studies were to determine the beetles' susceptibility to infection and subsequent death rate by exposure to three commercial strains of entomopathogenic fungi over time. Two different sets of bioassays (dipping beetles and dipping paper disks) were conducted to determine the time that each formulation of entomopathogenic fungi, *Beauveria bassiana* (BotaniGard® ES), *Isaria fumosorosea* (PFR 97® 20% WDG), and *Metarhizium brunneum* (Met F52 ES), takes to cause mortality of the ambrosia beetle species. The spore density of the fungal solutions in all bioassays was 2.4×10^6 spores/ml. The median survival time (ST₅₀) sequences for beetles dipped in the fungal suspensions were *Beauveria* < *Metarhizium* < *Isaria* for Xc and *Beauveria* = *Metarhizium* < *Isaria* for Xv. The ST₅₀ value sequence for both beetle species consuming treated filter paper disks was *Beauveria* = *Metarhizium* = *Isaria*. Results from field persistence of the fungal suspensions sprayed on avocado bolts demonstrated that conidia of *Beauveria* and *Metarhizium* persisted up to 21 days after treatment (DAT); more *Isaria* colony-forming units (CFUs) appeared 21 DAT than at 1 DAT. At 21 DAT, CFUs of *Isaria* were also found in plates of the *Beauveria* and control treatments. Field persistence trials after application of *Beauveria* or *Isaria* on avocado trees are ongoing; findings will be reported as available.

POSTER Wednesday 16:30 **MC-13**

Efficacy of *Metarhizium anisopliae* ICIPE 7 for the control of cattle tick

Paulin Nana^{1,2}, *Sunday Ekesi*¹, *Mercy Mumbi*³, *Nguya K. Maniania*¹

¹International Centre of Insect Physiology and Ecology, Nairobi, Kenya; ²Faculty of Agriculture and Agricultural Sciences, University of Dschang, Cameroon; ³Department of Veterinary Pathology, Microbiology and Parasitology, University of Nairobi, Kenya

Correspondence: pnaana@icipe.org

Ticks and tick-borne diseases cause serious economic losses to cattle industry. Current control strategies for these vectors rely on extensive use of chemical acaricides against which they have developed resistance. Entomopathogenic fungi are perceived as a promising alternative to acaricides. The present study evaluates the efficacy of *Metarhizium anisopliae* ICIPE 7 in controlling on-host ticks under field conditions. Twenty cows with an average of 437 ticks/animal were separated into four groups representing the following treatments: (1) Control; (2) Emulsifiable formulation of conidia (1×10^9 conidia ml⁻¹); (3) Amitraz and (4) Combination of Amitraz and fungus (1×10^8 conidia ml⁻¹ + 0.1% Amitraz). Each cow was sprayed once a week with two litres of the corresponding treatment in a crush. Tick load was recorded once per week. The persistence of conidia was also determined by sampling different parts of the skin of cattle using a cotton swab. Average number of ticks on cattle treated with fungus (133.4 ± 27.0) was significantly lower ($P < 0.05$) than the one in the controls (318.0 ± 60.4), representing 58.2% reduction. Treatment with Amitraz resulted in 79.5% reduction while 69.3% tick reduction was recorded in combination of fungus with Amitraz. Mortality of the ticks sampled from cows after spray applications ranged between 94-98% in fungus treatments against 3% in the controls. Viability of conidia varied according to animal body parts and ranged between 62.6 and 72.4% 7 days post-spray. These results indicate that *M. anisopliae* ICIPE 7 has potential for on-host tick control.

POSTER Wednesday 16:30 **MC-14**

Morphological and physiognomic study of seven isolates of an entomopathogenic fungus

E. C. Cavallo, J. A. Marfetan, P. J. Folgarait

Laboratorio de Hormigas, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Bernal, Buenos Aires, Argentina

Correspondence: patricia.folgarait@gmail.com

There is a need for non chemical agents to control leaf-cutting ants, among which are entomopathogenic fungi. Seven isolates of *Beauveria* spp have been previously tested on ants from seven *Acromyrmex lundii* nests and confirmed their virulence (half-life of the colony infected and recovery of the fungus from inoculated ants). In this study, morphological and physiognomic characteristics of these isolates were investigated with the aim to evaluate: a) if the isolates are different morphospecies; b) possible differences in their physiognomy and c) if the differences in virulence could be related by dissimilarities in physiognomy or morphotype. For this purpose, we measured twelve morphological variables in PDA medium, which are normally used to describe the *Beauveria* species. We also measured six physiognomic variables (colony size, height, color, texture, shape and symmetry) in PDA, Czapeck, Chitin and Caseinate medium. Morphological variables were tested using Cluster and PCA analyses while physiognomic variables were analyzed with PCA. The results obtained by studying the morphology, showed four different morphospecies, which could be explained by conidial diameter, morphology of conidogenous cells and colony color and diameter. When analyzing physiognomic variables, it was seen that all isolates behave differently, even within the same morphospecies. Moreover, when virulence data were studied, their ordination was not related with morphospecies either, but was correlated to some physiognomic variables such as colony size in PDA and Chitin. This implies that the entomopathogenic effect of the isolates was more dependent on their capacity to degrade carbon sources than to their morphotypes.

POSTER Wednesday 16:30 **MC-15**

Delivery of entomopathogenic fungi as a seed coating for control of soil-dwelling insect pests

Federico Rivas^{1,3}, *Trevor Jackson*², *John Hampton*¹, *Per Wessman*², *Jayanthi Swaminathan*², *Michael Rostas*¹, *Travis R. Glare*¹

¹Bio-Protection Research Centre, Lincoln University, Lincoln, New Zealand; ²AgResearch, Lincoln Research Centre, Lincoln, New Zealand; ³National Institute for Agricultural Research, Las Brujas, Uruguay

Correspondence: Federico.RivasFranco@lincolnuni.ac.nz

Recently, it has been shown that *Metarhizium* spp. can act as both insect pathogens and plant-growth-promoting agents. This bifunctional lifestyle of *Metarhizium* is illustrated by the expression of genes involved in the adhesion to insect (*mad1*) or plant surfaces (*mad2*). In addition, a plant carbon transporter, *Metarhizium* raffinose transporter (*mrt*), was reported as required for successful root colonization. The development of fungal entomopathogens as biocontrol agents requires an understanding of the interactions between fungi, insect pests and plants. Our present work is focused on the selection of entomopathogenic fungi to both colonize the plant rhizosphere and have biocontrol activity against insect pests when delivered through seed coating. In order to determine root competence, growth response of *Metarhizium* spp. isolates to maize root exudates was determined using microplate assays. Additionally, the presence of *mad1*, *mad2* and *mrt* was determined by PCR. Finally, the effects of *Metarhizium* spp. on maize plant growth and plant resistance

against larvae of the scarab beetle *Costelytra zealandica* was determined. The selection of root-competent strains is advisable for improve establishment and persistence of entomopathogenic fungi delivered as seed coating.

POSTER Wednesday 16:30 **MC-16**

Fat pellet strategy applied to the boll weevil (*Anthonomus grandis*) under laboratory conditions

Marcos Faria, Lorena K. B. Bravo, Bruna M. Kraus, Rogério B. Lopes

EMBRAPA Genetic Resources and Biotechnology, Brasília, DF, Brazil

Correspondence: marcos.faria@embrapa.br

An attract-and-kill approach based on fat pellets blended with an aggregation pheromone and conidia of entomopathogenic fungi was tested against adults of the boll weevil, *Anthonomus grandis* (Coleoptera: Curculionidae). In a preliminary screening assay, *A. grandis* from a rearing facility was immersed for 20 sec in 2-mL suspensions containing 6×10^8 viable conidia/mL of either *Beauveria bassiana* or *Metarhizium anisopliae*. Average confirmed mortality rates were $\leq 35.8\%$ for *B. bassiana* isolates and 61.7% for isolate CG46 of *M. anisopliae*. In another set of experiments, weevils were forced to contact fat pellets (without pheromone) impregnated with CG46 conidia, for either 30 min or 4 h at two incubation temperatures (26 or 30°C). The higher the exposure time and temperature, the higher the mycosis-confirmed mortality, reaching an average of 85.2% for the most favorable combination. Olfactometer assays revealed that fat pellets impregnated with both conidia and pheromone were highly attractive to weevils. Attraction was improved when other semiochemicals were added to pellets. Greenhouse experiments will be performed in 2015. The aim of this project is to develop an alternative strategy that could be tested under field conditions in the management of boll weevil populations.

POSTER Wednesday 16:30 **MC-17-STU**

Compatibility of fungicides with *Beauveria bassiana*

Sumanth S. R. Dara¹, Suchitra S. Dara², Alap Sahoo¹, Haripriya Bellam¹, Surendra K. Dara³

¹Stockdale High School, Bakersfield, CA, USA; ²Warren Junior High School, Bakersfield, CA, USA; ³University of California Cooperative Extension, San Luis Obispo, CA, USA

Correspondence: skdara@ucdavis.edu

Field studies in California demonstrated the potential of *Beauveria bassiana* in managing strawberry pests. Growers, however, are concerned about its compatibility with fungicides that are frequently applied to manage foliar diseases. To address this issue, pathogenicity of *B. bassiana* to *Tenebrio molitor* in the presence of eight fungicides from different mode of action groups that are commonly used for disease management in strawberries. Captan, Merivon, Microthiol Dispers, Pristine, Rally, Rovral, Switch, and Thiram were applied from 0 to 6 day interval prior to the application of *B. bassiana* and the mortality of *T. molitor* larvae were measured for six days. Captan and Thiram caused a significant reduction in the efficacy of *B. bassiana*. Remaining six fungicides did not affect *B. bassiana*. Time interval did not have any influence on any of the tested fungicides. Results address a major concern of grower by demonstrating that several fungicides are compatible with *B. bassiana* and support sustainable pest management practices.

POSTER Wednesday 16:30 **MC-18**

Characterization of *Tolypocladium cylindrosporium* (Hypocreales: Ophiocordycipitaceae) and its effectiveness in infecting *Aedes aegypti* eggs (Diptera: Culicidae)

Lina B. Flor-Weiler¹, Alejandro P. Rooney¹, Robert W. Behle¹, Daniel A. Strickman²

¹Crop Bioprotection Unit, Agricultural Research Service, U.S. Department of Agriculture, Peoria, IL 61604; ²(Retired), Office of National Programs, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD 20705

Correspondence: lina.weiler@ars.usda.gov or alejandro.rooney@ars.usda.gov

Availability of an effective egg-targeting system would allow for the control of a much greater portion of mosquito populations, because after emergence adults disperse away from egg-laying sites. Consequently, eggs form a reservoir of individuals highly susceptible to control measures. The purpose of this study was to evaluate the potential of the fungus *Tolypocladium cylindrosporium* as a control agent for the mosquito *Aedes aegypti*. Morphological characterization of *T. cylindrosporium* IBT 41712 was conducted and pathogenic activity of the fungus against *A. aegypti* eggs was evaluated. Fungal growth at different temperatures, conidia germination and sporulation on solid media was observed using light microscopy and scanning electron microscopy. The fungus formed white colonies turning creamy white when mature, and sporulation occurred after 8-10 days of incubation. The mycelia bore flask-shaped or swollen conidiophores producing smooth-walled, single-celled conidia that are oblong and cylindrical in shape when mature. Conidia had 96-98% germination after 10 days incubation and appeared to vary in size, ranging from 1µm to 2.5µm long. The optimum temperature for growth was 28 °C, with a maximum mean radial growth rate of 1.1 ± 0.02 mm/day. There was no fungal growth observed at 33, 37 or 40 °C. However, growth was observed at 4, and 12 °C, albeit much more slowly than at room temperature or optimal levels. Significant reduction in larval hatch was observed for fungus treated eggs with significantly fewer larvae surviving (20%) at 14 days post treatment compared with those surviving in the control (93%).

POSTER Wednesday 16:30 **MC-19**

Stress-induced changes in the dopamine levels of haemolymph of cabbage armyworm *Mamestra brassicae* and Colorado potato beetle *Leptinotarsa decemlineata*

E. A. Chertkova, O. N. Yaroslavtseva, I. M. Dubovskiy, V. V. Glupov
Institute of Systematics and Ecology of Animals, Russian Academy of Sciences, Siberian Branch, Frunze str. 11, Novosibirsk 630091, Russia

Correspondence: chertkaterina@yandex.ru

We've studied effect of several stress factors on dopamine levels in haemolymph of cabbage armyworm *Mamestra brassicae* and Colorado potato beetle *Leptinotarsa decemlineata*. It was detected the increase of dopamine concentration in haemolymph of *M. brassicae* in 7 times against to control under high temperature (50°C) treatment. The mechanical injury (burn) led to increase of dopamine concentration in haemolymph of *M. brassicae* in 5.5 times against to control. We've found that *Beauveria bassiana* fungal infection resulted to 4 times rising of dopamine concentration in haemolymph of *M. brassicae*. Toxigenic strain of *Metarhizium robertsii* induced the enhancing dopamine level in haemolymph of *Leptinotarsa decemlineata* in 70 times compared with control and in 2.5 times against to biotrophic strain *M. robertsii*. Presented data show that dopamine

is one of the key stress molecule and its level can be important in immune defence against entomopathogenic fungi.

POSTER Wednesday 16:30 **MC-20**

Biosynthesis of silver nanoparticles using the fungus *Trichoderma viride* for the toxicity on *Aedes aegypti* mosquito and their antibacterial activity

Siva Kamalakannan, Chandrakasan Gobinath, Kadarkarai Murugan

Unit of Medical Entomology, Department of Zoology, Bharathiar University, Coimbatore-641046, India

Correspondence: kamal410@yahoo.com

Silver nanoparticles are explored in recent years as an alternative approach to effectively kill mosquito vector and drug resistant pathogenic microorganisms. In the present study, an eco-friendly process for the synthesis of nanoparticle, using a fungus (*Trichoderma viride*) has been attempted. The fungus biomass supernatant was used for the biosynthesis of Ag-NPs. The aqueous silver ions (Ag⁺) were reduced to silver metal nanoparticles (Ag m-NPs), when treated with the fungal supernatant. After 24h of treatment, silver nanoparticles (Ag-NPs) were obtained. These Ag-NPs were characterized by UV-Vis, Scanning Electron Microscopy (SEM) with Energy Dispersive Spectroscopy (EDS) and X-ray Diffraction (XRD) were used to identify these NPs. The nanoparticles exhibited maximum absorbance peak at 420 nm in UV-Vis spectroscopy. The NPs surface morphology revealed from SEM images shows formation of well-dispersed Ag-NPs of 50 nm, and the presence of silver was confirmed by EDX and Fourier transform infrared spectroscopy (FTIR) analysis. The efficacy of mycosynthesized AgNPs tested concentrations of 5, 10, 15, 20 and 25 ppm against L1 to L4 instar larvae and pupae of *Aedes aegypti*; LC50 (LC90) values are 13.15(24.55); 12.08(29.45); 10.47(36.65); 9.85 (41.31) and 9.72 (42.24) in larvae and LC50 (LC90) values was 5.09 (15.53) in pupae, respectively. The mortality rates were positively correlated with the concentration of AgNPs. The microbes selected for the present study for the antibacterial activity were *E. coli*, *P.aeruginosa*, *B.cereus*, *Enterococci*, *E.aerogens*. We conclude that the nanoparticle synthesized from the fungus has great potential mosquito larvicidal agents as well as antimicrobial compound against pathogenic microorganisms.

Microsporidia

POSTER Wednesday 16:30 **MI-1**

Effects of the microsporidian pathogen, *Nosema adaliae* on the seven-spotted lady beetle, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae)

Elkibir Entisar, Bjornson Susan

Department of Biology, Saint Mary's University, 923 Robie Street, Halifax, NS B3H 3C3 Canada

Correspondence: Susan.Bjornson@smu.ca

There has been a noticeable decline in lady beetles in North America over the past few decades. Because lady beetles play an important role in the regulation of aphids and other soft-bodied insect populations in nature, lady beetle population decline has become a significant cause for concern in agriculture systems. Decreased populations are attributed to several causes, including competition from more aggressive lady beetle species (such as the multi-coloured Asian lady beetle, *Harmonia axyridis* Pallas) and the potential impact of insect pathogens. Microsporidia are fungal

pathogens that commonly infect lady beetles. Recent studies have focused on the prevalence and potential impact of microsporidia in lady beetles, including the effects of microsporidian pathogens on host development and host specificity. The objective of the study is to gain knowledge about the effect of the microsporidian pathogen *Nosema adaliae* from the two-spotted lady beetle *Adalia bipunctata* L. on the development of the seven-spotted lady beetle, *Coccinella septempunctata* L. An examination of the effects on development and the impact of the pathogen on second-generation beetle larvae could provide insight to the population dynamics of *C. septempunctata*. The results from this study will provide more information to understand the effects of pathogen on host development, fecundity, and vertical transmission.

POSTER Wednesday 16:30 **MI-2**

Online weather-based risk forecast model for *Nosema* spp. infections in German honey bee apiaries and projected risk under climate change

Anto Raja Dominic¹, Sebastian Gisder², Elke Genersch², Andreas Linde¹

¹Hochschule für nachhaltige Entwicklung Eberswalde, Dept. of Forest and Environment, Alfred-Möller-Str. 1, 16225, Eberswalde, Germany; ²Länderinstitut für Bienenkunde Hohen Neuendorf e.V., Friedrich-Engels-Str. 32, 16540, Hohen Neuendorf, Germany

Correspondence: Anto@zedat.fu-berlin.de

Amidst wide-spread concerns of the loss of honeybee colonies around the world in the last decades, the increasing spread of the microsporidian *Nosema ceranae* was cause for alarm. The consequences of *N. ceranae* infections in colonies concerning honeybee health are discussed controversially. However, it has been shown that *N. ceranae*, originally a pathogen of the Asian honeybee, has already replaced *N. apis*, a native pathogen of the European honeybee, in many parts of the world. Laboratory studies have conclusively shown that *N. ceranae* is better adapted to warmer weather and is sensitive to close to freezing temperatures.

With this background, we analyzed the relationship between local weather conditions and *Nosema* spp. prevalence in the apiaries of Northeastern Germany. The prevalence data come from a cohort study of 44 apiaries in autumn and early spring over a period of 10 years from 2005-2014. The weather variables that best described the relationship were tested and found reliable for their predictive performance on (pseudo) new data.

We used the selected variables to forecast risk of *Nosema* spp. infections for 6 of the federal States of Northeastern Germany. This risk map is available as an online tool to help the beekeepers to monitor their colonies and take precautionary measures to prevent disease outbreak and further spread of the infection. Due to strong correlation between prevalence and temperature, we also project future prevalence of *Nosema* spp. under different climate change scenarios for the next 60 years.

POSTER Wednesday 16:30 **MI-3**

rRNA organization in a new light: A microsporidium infecting mountain pine beetle, *Dendroctonus ponderosae* (Coleoptera: Scolytidae)

George Kyei-Poku, Debbie Gauthier

Natural Resources Canada, Canadian Forest Service, Great Lakes Forestry Centre, 1219 Queen Street East, Sault Ste. Marie, Ontario, Canada P6A 2E5

Correspondence: gkyeipok@nrcan.gc.ca

There is enormous interest in the evolution of microsporidian genomes and understandings gained from such studies may help decipher the plausibility of various scenarios and shed light on the evolutionary origin of reorganized rRNA in microsporidia. The rRNA cistron of microsporidia is traditionally arranged in the order small subunit-internal transcribed spacer-large subunit, which conforms to the typical arrangement of these gene units in almost all organisms. We found a rearrangement of the cluster in a microsporidium infecting mountain pine beetle (MPB) where the large subunit precedes the small subunit (reverse organization of the rRNA subunits; LSUrRNA-ITS-SSUrRNA-IGS-5S) as has previously been reported for several species in the microsporidian genus *Nosema* and a species, *Glugoides intestinalis*. We provide evidence that the arrangement reported in the microsporidium infecting MPB is a third, independent event occurring in the microsporidia species with reverse organization of the rRNA subunits and may have taxonomic implications for members of the genus *Nosema*.

POSTER Wednesday 16:30 **MI-4**

Light and electron microscopic observations of *Anncaliia* sp. (Microsporidia: Tubulinosematidae) from *Dikerogammarus villosus* (Amphipoda: Gammaridae)

Yuri S. Tokarev¹, Yuliya Y. Sokolova^{2,3}, Irma V. Issi¹

¹Institute for Plant Protection; ²Institute of Cytology, Russian Academy of Sciences, St. Petersburg, Russia; ³School of Veterinary

Medicine, Louisiana State University, Baton Rouge LA, USA

Correspondence: sokolova@lsu.edu

Among 100 specimens of Killer Shrimp *Dikerogammarus villosus* captured in 2013 at the intertidal zone of the Azov sea, five were infected with a microsporidium producing masses of elongate binucleate spores within hypertrophied inner tissues. Ultrastructural analysis showed disporoblastic sporogony and diplokaryotic organization of the nucleus in all developmental stages. The additional surface layer of the sporogonial developmental stages was composed of tubular extensions, translucent vesicles and electron-dense granules. The spores possessed a two-layered exospore and thick endospore. The polaroplast was bipartite, its anterior and posterior parts were made of rare lamellae and thick tubular structures, respectively. The diplokaryon was surrounded by 2-4 layers of endoplasmic reticulum, encrusted with abundant ribosomes. The polar tube was slightly anisofilar, possessing between 13 and 18 coils arranged in one layer with 1-3 distal coils of lesser diameter. The ultrastructural characters correspond to those of the genus *Anncaliia* and molecular phylogenetic study supports this diagnosis. This is a first case of a natural infection of crustaceans with an *Anncaliia* species, though experimental infection via injection was demonstrated in Decapoda for *Anncaliia algerae* (Undeen, Maddox, 1973, J. Invertebr. Pathol., 22:258-265).

Nematodes

POSTER Wednesday 16:30 **NE-1**

Improved survival time of infective juveniles of *Steinernema glaseri* collected on Paris plaster

C. I. Cortés-Martínez¹, J. Ruiz-Vega¹, E. E. Lewis²

¹Instituto Politécnico Nacional, CIIDIR-Oaxaca, Oaxaca, 71230, México; ²Department of Nematology, University of California,

Davis, Davis, CA 95616, USA

Correspondence: solemia7@hotmail.com

To evaluate the survival time and infectivity of *Steinernema glaseri* Infective Juveniles (IJ's) mechanically pelletized in Celite® 209 brand diatomaceous earth (DE), IJ's of *Steinernema glaseri* (strain NJ-43) were reproduced in larvae of *Galleria mellonella* and subjected to two collect treatments, namely: White traps (WT) and plaster of Paris (PP). The IJ were collected 3 days after the onset of the emergency and a prototype machine was used to produce the pellets. IJ's in bi-distilled water suspension (AS) was the control. The formulations were stored at temperature of 24 ± 5 °C and relative humidity of 96 ± 3 %. Moisture content (MC) of the pellets was determined daily. The counting of live and dead IJ's was performed every two days, while infectivity over time was tested by exposing third instar *Phyllophaga vetula* larvae to IJ's every two days. The bioassay was replicated 5 times. Survival analysis was performed using Kaplan-Meier graphs and Log-rank test. Initial MC of pellets containing IJ's collected in WT was 47%, while in pellets with IJ's collected on PP initial WC was 50%. The median survival time (MST) was 23.0 and 34.5 days, respectively. However, the MST of the control treatment was 28.12 days. *P. vetula* larvae were little susceptible to infection by IJ stored as WS, but somewhat susceptible to infection by IJ formulated in DE. About 2% of total *P. vetula* larvae exposed to IJ's collected from PP were infected, while only 1.2% of the larvae exposed to IJ's from WT were infected.

POSTER Wednesday 16:30 **NE-2**

Ecological characterization of *Steinernema siamkayai* (Rhabditida: Steinernematidae), a warm-adapted entomopathogenic nematode isolate from India

Ramalingam Karthik Raja^{1,2}, Aishwarya Dilipkumar¹, Sivaperumal Sivaramakrishnan³, Selcuk Hazir², Pachiappan Perumal¹

¹Department of Biotechnology, Periyar University, Salem, Tamil Nadu, India; ²Faculty of Arts and Science, Department of Biology, Adnan Menderes University, 09010 Aydin, Turkey; ³Department of Biotechnology and Genetic Engineering, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India

Correspondence: sriramkarthik83@gmail.com

Our study describes basic ecological properties of *Steinernema siamkayai* Tiruchirappalli strain from India. The effect of temperature on nematode infectivity and development, laboratory host range and foraging behaviour were determined. The data showed that *S. siamkayai* is a warm-adapted nematode species with larval mortality observed between 15°C and 37.5°C and nematode reproduction occurring between 20°C and 35°C. All insect species used in this study were susceptible to *S. siamkayai* under laboratory conditions. Sixty infective juveniles (IJs) per insect were used and the lepidopterans, *Galleria mellonella* (100%) and *Spodoptera exigua* (85%), were the most susceptible species followed by the dipteran, *Ceratitis capitata* (60%), and lepidopteran, *Cydia splendana* (55%), and the coleopteran, *Tenebrio molitor* (45%), whereas the coleopteran, *Curculio elephas* (25%), was the least susceptible species. *S. siamkayai* infective juveniles (IJs) stood on their tails and jumped and could also attach to a mobile host at a rate of 27 IJs larvae-1 out of 1000 IJs in 10 min. Larval mortality of *G. mellonella* by *S. siamkayai* on different substrates (sand, filter paper, filter paper sprinkled with sand) was 100% on all substrates. Number of IJs out of 100 IJs that penetrated into a *G. mellonella* host at different soil depths was the highest at the surface (44 IJs larva-1) and the lowest at 5 cm depth (13 IJs larva-1) with no larval mortality observed at 10 cm depth. In addition, the symbiotic bacterium of *S. siamkayai* was identified as *Xenorhabdus stockiae* based on genotypic and phenotypic characterisation. Bacterial growth was observed between 15°C and 41°C.

POSTER/ Wednesday 16:30 **NE-3****Evaluation of entomopathogenic nematodes *Steinernema feltiae* and *Heterorhabditis bacteriophora* against melon aphid (*Aphis gossypii* Glow, Hemiptera Aphididae)**

Nana Mikaia

Department of Natural Sciences and Health Care. Sokhumi State University, 9, Anna Politkovskaya Str. Tbilisi 0186, Georgia
Correspondence: nonamikaia@gmail.com

The aim of this study was to determine the biological control effect of entomopathogenic nematodes *Steinernema feltiae* and *Heterorhabditis bacteriophora* on melon aphid *Aphis gossypii* in the laboratory conditions. The experiments were conducted in 10 cm Petri dishes lined with a moistened filter paper. One infested cucumber plant leaf containing approximately 120-150 *A.gossypii* was placed in each Petri dish and the *S.feltiae* and *H.bacteriophora* nematodes were applied with concentrations of 0,500,1000 and 1500 infective juveniles/ml. Insect mortality was checked 3, 5 and 7 days after the treatment. The results showed that *S.feltiae* was with higher virulence against *A.gossypii*, than *H.bacteriophora* and the mortality was related to time, nematode type and concentrations. Seven days after the treatment, 500, 1000 and 1500 IJs/ml applications exhibited 20, 58 and 78% mortality for *S.feltiae*, and respectively 15, 28, and 46% for *H.bacteriophora*. In conclusion was determined that *A.gossypii* can be controlled by *S.feltiae*, better than *H.bacteriophora*, but further studies should be conducted in greenhouse and filed conditions.

POSTER Wednesday 16:30 **NE-4-STU****A new way for number regulation of South American tomato moth, *Tuta absoluta* in Georgia**

Mariam Chubinishvili, Tsisia Chkhubianishvili, Manana Kakhadze, Iatamze Malania, Rusudan Skhirtladze, Irine Rizhamadze

Biological Control Laboratory, Kanchaveli Institute of Plant Protection, Agricultural University of Georgia
Correspondence: m.chubinishvili@agrundi.edu.ge

The new local entomopathogenic nematode - *Steinernema feltiae* Georgian strain „Geo-nema“ is considered as a significant biological agent for number regulation to South American tomato moth, *Tuta absoluta* (Lepidoptera: Gelechiidae). At present this invasive pest insect causes great damage (80-90%) on tomato plants in greenhouses of Georgia. I-II instars larvae live on leaf surface at development stage. The pest pupation takes place in the soil or within mines of leaf surface. Where the relationship of “host-pathogen” is established by spray nematode suspension on tomato plants, or the soil treatment. The susceptibility of local entomopathogenic nematodes – *S.feltiae* to *T.absoluta* in laboratory and greenhouse conditions were established. Determination of invasive ability of *S.feltiae* has conducted according to recent methods in insect Nematology. The different instars of pest larvae were settled on tomato plants pot culture and were treated with nematode suspension - 500 IJs/ml for invasion at special container at 23-25°C and 60- 70% RH laboratory conditions. The application was carried out two times with 7 days interval and a sterile water was used in control variants. The infected larvae were detected after 48, 72, 96 hr. Data was analysed with two way ANOVA (p=0.05). The pest mortality index by “Geo-nema” suspension was achieved to 79.2%. The experiments and observations at the stationary plot (200-250 sq.m greenhouse (village Misaktsieli, Mtskheta region) was planted by Dutch tomato sort - “Rose pinkinikum” were conducted twice with the application rate 25 million IJs/ha. The

biological efficacy of nematode suspension achieved to 53.2 – 54.1%.

POSTER Wednesday 16:30 **NE-5****Roots exudates favor friends and hinder foes in the rhizospheric nematode community**

I. Hiltbold¹, G. Jaffue², T. C. J Turlings²

¹Hawkesbury Institute for the Environment, University of Western Sydney, Penrith, NSW, Australia; ²FARCE lab, University of Neuchâtel, Neuchâtel, Switzerland

Correspondence: i.hiltbold@uws.edu.au

Root tips exude a large amount of photosynthetic assimilated carbon. Some of these organic compounds are detrimental for root pest organisms whereas some can favor beneficials. Here we tested the effect of pea root cap exudates on three nematodes species commonly found in the rhizosphere. *Heterorhabditis bacteriophora* is an entomopathogenic nematode (EPN) commonly used as a biological control agent against soil-dwelling arthropods. The soybean cyst nematode (SCN) *Heterodera glycines* and the root-knot nematode (RKN) *Meloidogyne incognita* are plant-parasitic nematodes causing severe damage to root systems; they are considered major agricultural pests. Pea root cap exudates induced a reversible stage of quiescence to the three tested species. In the presence of diluted exudates, which reflected concentrations more likely encountered in the environment distal from the root tip, EPNs exhibited a significant increase in the number of oscillations per minute. In contrast, SCN and RKN activity was significantly reduced when the nematodes immersed in the dilution of pea root exudates. When applied with diluted root exudate, EPN showed higher infectiousness than in water. These results suggest that the same compound (or blend of compounds) can simultaneously favor beneficial organisms and hinder pests. Identifying the molecules involved and understanding the mechanisms leading to dramatically different effects on nematodes can help to develop ecologically sound methods to manage both insects, by favoring their natural enemies, and nematode pests, by limiting their activity in the rhizosphere.

POSTER Wednesday 16:30 **NE-6-STU****Host searching behavior of entomopathogenic nematode from the subarctic zone in Japan under low temperature**

Yohsuke Maguchi¹, Toyoji Yoshiga², Daigo Aiuchi², Masanori Koike¹

¹Department of Agro-environmental Science, Obihiro University of Agriculture & Veterinary Medicine, Obihiro, Japan; ²Laboratory of Nematology, Department of Applied Biological Sciences, Faculty of Agriculture, Saga University; ³Research Center for Global Agro-medicine, Obihiro University of Agriculture and Veterinary Medicine, Japan

Address for Correspondence: koike@obihiro.ac.jp

Entomopathogenic nematodes (EPNs) are used as biological control agent against Lepidoptera and Coleoptera in Japan. But utilization of EPN is little in the subarctic zone in Japan. The reason is decrease of pathogenicity symbiosis bacteria and host searching behavior by low temperature. But few studies are available on host finding behavior under low temperature. Also the cause of decrease of host finding behavior under low temperature is mostly unknown. So this study investigated the effect of low temperature against host searching behavior and host attractiveness for odors. First, we isolated EPNs from several soils in Hokkaido, Japan, under laboratory conditions. Next, host finding behavior and host attractiveness for host odors of five EPN

strains were isolated in Hokkaido against last instar larvae of wax moth, *Galleria mellonella* was evaluated in the laboratory at 10°C, 15°C and 20°C. All strains were observed active host searching behavior and host attractiveness for host odors at 15-20°C. *Hksf11s* was the only strain that observed active host searching behavior and host attractiveness for host odors. These result suggested that the decrease of host searching behavior under low temperature may be related to host attractiveness for host odors.

POSTER Wednesday 16:30 **NE-7**

Genomic determinants of the entomopathogenic lifestyle from independently arising nematodes

Hillel Schwartz¹, Adler Dillman², Paul Sternberg²

¹HIMI and Division of Biology and Biological Engineering, California Institute of Technology, Pasadena CA 91125, USA;

²Department of Nematology, University of California Riverside, CA 92521, USA

Correspondence: hillels@caltech.edu

Entomopathogenic nematodes disperse as infective juveniles to hunt their insect prey. Upon finding a host, they invade its body and release symbiotic pathogenic bacteria that rapidly kill the insect and convert it into a food source, while preventing colonization of the carcass by other microorganisms and predation by other scavengers. This lifestyle has arisen independently at least three times, in two different clades: the *Steinernema* and of *Heterorhabditis* nematodes, and *Oscheius carolinensis*.

To better understand how these nematodes have converged on this shared strategy, we have sequenced the genomes and transcriptomes of five species of *Steinernema*, four species of *Heterorhabditis*, and of *O. carolinensis*. We are seeking genomic traits common to these nematodes but not shared with free-living or with parasitic nematodes, some of them more closely related to these EPNs but not sharing their symbiosis. Comparisons of these species may reveal mechanisms that regulate responses to bacterial interactions and variations that correlate with differences in lifestyle or bacterial compatibility.

In addition to genomic studies of the different EPN species, we are developing *H. bacteriophora* as a laboratory organism. *H. bacteriophora* grows well on plates, is reportedly susceptible to RNAi and transgenesis, and can develop as selfing hermaphrodites, and so should be a powerful system for molecular genetic study of symbiosis. When cultured at low density these nematodes develop almost exclusively as females. We have screened for and isolated a constitutively hermaphroditic mutant for use in molecular genetic studies of symbiosis and of sex determination.

Viruses

POSTER Wednesday 16:30 **VI-1-STU**

The impact of virus diversity on the evolution of pest resistance

Leon Yu Zheng Li¹, Jenny S. Cory¹

¹Department of Biological Sciences, Simon Fraser University, Burnaby, BC, Canada

Correspondence: leonl@sfu.ca

Pathogen mixtures have long been theorized to reduce the rate of evolution of resistance in host. The goal of this project is to determine whether virus diversity affect the rate of evolution of resistance in pests. Wild-type virus containing mixed variants have been shown to more pathogenic possibly due to varying modes of

infection. We hypothesize that there is a decrease in the rate of resistance evolving as virus diversity increases. Using the cabbage looper (*Trichoplusia ni*) as a model, we have subjected *T. ni* to multigenerational selection for resistance using single and mixed variants of *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV). The selection experiment is currently ongoing and we will present the progress made so far. The results will not only provide information on the effect that multiple selection pressure can have on the rate of evolution, but also give us insight into better pest management practices. This better understanding of microbial insecticide usage will allow us to utilize these natural control agents more effectively in the future.

POSTER Wednesday 16:30 **VI-2**

Constitutive and herbivore-induced defenses of soybean inhibits baculoviral disease in the fall armyworm, *Spodoptera frugiperda*

Ikkei Shikano, Kelli Hoover

Department of Entomology, Pennsylvania State University, University Park, PA, USA

Correspondence: kxh25@psu.edu

Plants play an important role in the evolution of insect-pathogen interactions. Inter- and intraspecific variation in host plant chemistry can strongly influence the susceptibility of caterpillars to baculovirus infection. When baculoviruses are ingested with foliage, plant defenses can interfere with fatal infection by the virus. Here, we investigated the impact of constitutive and induced defenses in soybean on the fitness of *Spodoptera frugiperda* MNPV (SfMNPV) infecting larvae of the fall armyworm (FAW), *S. frugiperda*. Soybean genotypes vary markedly in their constitutive and inducible defenses against insect herbivores and we are currently testing 9 different genotypes for levels of plant defenses including protease inhibitors, lypogenase, and phenylalanine ammonia lyase and the impacts of these defenses on mortal virus infection. We applied SfMNPV to leaf disks of soybean genotypes differing in levels of constitutive defenses, and found that FAW were less susceptible when SfMNPV was ingested on soybeans that have high constitutive defenses. To determine the impact of induced soybean defenses on SfMNPV infectivity, we will induce soybeans by exogenously applying jasmonic acid, a phytohormone that upregulates plant defenses against chewing herbivores. We predict that SfMNPV ingested with induced foliage will result in lower mortality in FAW, fewer virus progeny and longer time to death, thereby resulting in reduced fitness for the virus. While plant defenses are beneficial for plants in terms of reduced herbivore feeding and growth, our study suggests that plant defenses could negatively impact beneficial microbes that act as potential 'bodyguards' for plants.

POSTER Wednesday 16:30 **VI-3-STU**

Three baculoviruses infecting white satin moth (*LesanPV*), douglas-fir tussock moth (*OpMNPV*) and pale tussock moth (*DapuNPV*) cluster together on the phylogenetic tree

Martyna Krejmer¹, Lukasz Rabalski¹, Iwona Skrzeczek², Jadwiga Ziemnicka³, Boguslaw Szewczyk¹

¹Intercollegiate Faculty of Biotechnology University of Gdansk and Medical University of Gdansk, Department of Recombinant Vaccines, Gdansk, Poland; ²Forest Research Institute, Department of Forest Protection, Sekocin Stary, Poland; ³Institute of Plant Protection, Department of Biological Control and Quarantine, Poznan, Poland

Correspondence: martyna.krejmer@biotech.ug.edu.pl

The white satin moth *Leucoma salicis* L. (Lepidoptera, Lymantriidae) is a dangerous defoliator occurring mainly in

Europe and Asia, but it was also introduced to North America in 1920s. The caterpillars feed principally on the leaves of poplar, cottonwood, aspen and willow trees (family Salicaceae) and less commonly on oak. They attack healthy trees, what make them the primary pest and can lead to massive defoliations and weakened trees. One of the main natural enemies of this insect is baculovirus, LesaNPV (*Leucoma salicis* Nucleopolyhedrovirus). For population control of white satin moth the biopesticides based on LesaNPV baculovirus can be used. This type of control agents are claimed to be very safe for human and animals, because they do not infect organisms other than arthropods and cannot replicate outside their natural host. It has been reported in the literature that LesaNPV is infective against the close relative of its host, *Orygia pseudotsugata* L., but OpMNPV does not infect *Leucoma salicis* L. According to short nucleotide sequences of three genes (polh, lef-8, pif-2) available in the NCBI database LesaNPV was annotated as Alphabaculovirus group I (Jakubowska et al., 2005). In our studies we have prepared the draft of full genome of LesaNPV which we found highly similar to genome of OpMNPV and DapuNPV. We identified 153 ORFs (open reading frames) in LesaNPV genome, few were not present in OpMNPV (like *he65*) or were fused from two OpMNPV ORFs (Op82-Op83). The phylogenetic relationship of LesaNPV and other baculoviruses will be presented.

POSTER Wednesday 16:30 **VI-4**

Screening field collected Lepidoptera larvae for new virus isolates

Michelle T. Franklin¹, Amy Huang¹, Yan Han¹, Matilda Tabert¹, Martin Erlandson², Stefan Richard³, Deborah Henderson¹

¹Institute for Sustainable Horticulture, Kwantlen Polytechnic University, Langley, BC, Canada; ²Saskatoon Research Centre, Agriculture and Agri-Food Canada, Saskatoon, SK, Canada; ³Sylvia Technologies Inc., Fredericton, New Brunswick, Canada

Correspondence: Michelle.Franklin@kpu.ca

Alpha and Betabaculoviruses that target lepidopteran larvae remain underutilized as biopesticides, despite their suitability for organic farming and high level of safety for humans and beneficial arthropods. In Canada many economically important brassica crops are grown organically, however lepidopteran pests such as diamondback moth (*Plutella xylostella*), cabbage looper (*Trichoplusia ni*), and imported cabbageworm (*Pieris rapae*) pose a serious threat to these crops. The development of resistance to biologically based products such as *Bacillus thuringiensis* (Bt) and spinosad have reduced the number of products available to growers for control of these pests. Baculoviruses could offer an effective alternative control for these pests in organic brassica crops. To detect local baculovirus strains, we collected and froze over 3000 larvae of these three species from 17 farms throughout the southwest and interior of British Columbia in 2014. Additional larval collections will take place during the summer of 2015. Larvae from each site collected in 2014 were homogenized and we are beginning to perform assays where homogenized larvae are spread on the leaf surface and fed to healthy second instar larvae of the same species. Mortality is assessed daily and cadaver smears will be examined for the presence of virus particles using microscopy. Cadavers that show signs of viral infection will be fed to healthy larvae to further amplify new isolates and molecular identification will be undertaken for promising isolates. Detection of virus isolates is the first step in the development and commercialization of new baculovirus products and in future could provide growers with new biopesticides.

POSTER Wednesday 16:30 **VI-5-STU**

***Culex pipiens* - associated Tunis virus: A new mosaic virus in common house mosquitos**

Diane Bigot¹, Elisabeth A. Herniou¹, Marion Ballenghien², Mylène Weill², Célestine Atyame², Nicolas Galtier², Philippe Gayral¹

¹Institut de Recherche sur la Biologie de l'Insecte, UMR 7261, CNRS, Université François-Rabelais, 37200 Tours, France; ²Institut des Sciences de l'Evolution de Montpellier, UMR 5554, CNRS, Université Montpellier 2, Place E. Bataillon, 34095 Montpellier, France

Correspondence: diane.bigot@etu.univ-tours.fr

Many viral epidemics emerge from previously unknown viruses. The study of novel potential reservoirs of new viruses could help anticipating such viral emergence. In this perspective mosquitos are good reservoir candidates because they are efficient viral vectors. Twenty-two common house mosquitos (three species of the *Culex* genus) were sampled worldwide in the fields. Based on individual mRNA extraction, transcriptomes were obtained to by Illumina sequencing technology, representing 690 million reads. A homemade bioinformatics pipeline was used to characterise potential new complete viruses in these transcriptome data. Two variants of a new RNA virus were found in *Culex pipiens* mosquitos sampled in Tunisia and their genomes consisted in single-stranded RNA of 6.8kb in size. These two viral sequences referred as *Culex pipiens* Associated Tunis Virus (CpATV) strain Moknine and strain ElHabibia, shared 99.6% nucleotide identity. They were independently sampled six years apart, in 2005 and in 2011. CpATVs are composed of four ORFs, one ORF contained a helicase and a RNA-dependant RNA polymerase domain related to a mosquito Negevirus, two ORFs contained capsids protein domains related to *Virgaviridae* (plant viruses) among others. These two CpATVs displayed a mosaic genomic organisation with complex phylogenetic origins, and might therefore belong to a new virus family. Still, the true host or the life cycle of CpATVs is not known at this stage.

POSTER Wednesday 16:30 **VI-6**

Newly sequenced full genome of Nun moth (*Lymantria moanacha*) baculovirus show its high similarity to gypsy moth (*Lymantria dispar*) baculovirus

Lukasz Rabalski¹, Martyna Krejmer², Iwona Skrzecz², Bartosz Wasag³, Boguslaw Szewczyk¹

¹Intercollegiate Faculty of Biotechnology University of Gdansk and Medical University of Gdansk, Department of Recombinant Vaccines, 80-822 Gdansk, Kladki Str. 24, Poland; ²Forest Research Institute, Department of Forest Protection, 05-090 Raszyn, Braci Lesnej Str. 3, Sekocin Stary, Poland; ³Medical University of Gdansk, Department of Biology and Genetics, 80-211 Gdansk, Debinki Str. 1, Poland

E-mail address: lukasz.rabalski@biotech.ug.edu.pl

Nun moth (*Lymantria moanacha*) belonging to Lepidoptera, Lymantriidae is currently one of the most important pest of conifers in Poland. Caterpillars of this moth attack older and healthy pines, spruces and larches, but is also able to feed on deciduous trees. Nun moth is widely present throughout Europe. The natural biological control of this pest population is provided by *Lymantria monacha* Nucleopolyhedrovirus (LymoNPV) from *Baculoviridae* family. To date little is known about LymoNPV genome and phylogenetic relationship to others baculoviruses. To get better insight into LymoNPV molecular biology and its genetic correlation with other baculoviruses we isolated, sequenced on next generation platform and annotated full genome of Polish LymoNPV strain. In this presentation, after detailed DNA

sequencing and phylogenetic analysis, we show how LymoNPV is related to previously sequenced baculoviruses.

POSTER Wednesday 16:30 **VI-7**

Comparison among betabaculovirus isolates from Gelechiidae insect family

Gloria P. Barrera¹, Juliana A. Gómez², Mariano N. Belaich², Pablo D. Ghiringhelli², Laura Villamizar¹

¹Corporación Colombiana de Investigación Agropecuaria, Corpoica, Bogotá, Colombia; ²Laboratorio de Ingeniería Genética y Biología celular y Molecular, Área Virosis de Insectos (LIGBCM-AVI), Dto. De Ciencia y Tecnología, Universidad Nacional de Quilmes, Provincia de Buenos Aires, Argentina
Correspondence: gbarrera@corpoica.org.co

Tecia solanivora (Povolny) and *Phthorimaea operculella* (Zeller) are insect species belonging to the potato tuber moth complex (Lepidoptera: Gelechiidae) and *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is considered an important pest of tomato crop. Two Colombian isolates of granulovirus (GVs) recovered from larvae of *T. solanivora* and *T. absoluta* and one isolate of granulovirus of *P. operculella* were subjected to molecular and morphological analysis. Molecular identification and phylogenetic analysis of Colombian granulovirus isolates were performed using the combined complete sequence of highly conserved baculovirus genes: *late expression factor 8* (*lef-8*), *late expression factor 9* (*lef-9*) and *granulin* (*gran*). The genetic distances of these concatenated genes for species demarcation showed that the K-2 P distance among *T. solanivora* GV, *T. absoluta* GV and *P. operculella* GV was inferior to 0.015 indicating that they belongs to the same baculovirus specie. Additionally, the restriction endonuclease analysis (REN) using six enzymes showed similar patterns among GV's isolates from different insect species. Electron transmission images showed similar morphologies of OBs with ovoidal shape containing a single virion. *T. absoluta* GV OBs had the largest average size between 514 nm x 249 nm, while the average sizes for *T. solanivora* GV and *P. operculella* GV OBs were 448 nm x 252nm and 158nm x 224 nm, respectively. The results showed very similar isolates from Gelechiidae insect family. This could be due to the geographical proximity of where isolates were recovered and the possible presence of the three insects host in the same crop.

POSTER Wednesday 16:30 **VI-8**

Genome sequence analysis of a two alphabaculoviruses and a betabaculovirus from armyworms of genus *Mythimna*

Robert L. Harrison¹, David A. Theilmann², Martin A. Erlandson³

¹USDA Agricultural Research Service, Beltsville, MD, USA; ²Agriculture and Agri-Food Canada, Summerland, BC, Canada; ³Agriculture and Agri-Food Canada, Saskatoon, SK, Canada
Correspondence: Robert.L.Harrison@ars.usda.gov

Noctuid species of the armyworm genus *Mythimna* Guenée are widespread pests of graminaceous crops and pastures. Baculoviruses have been identified from species of *Mythimna*, and the genomes of an alphabaculovirus from *Mythimna* (*Leucania*) *separata*, LeseNPV, and a betabaculovirus from *Mythimna* (*Pseudaletia*) *unipuncta*, PsunGV-H, have been sequenced. To further characterize the genetic diversity of baculoviruses from *Mythimna*, we isolated genomic DNA from the occlusion bodies of virus isolates *Mythimna* (*Pseudaletia*) sp. NPV #7 (MyspNPV#7) and *Mythimna* (*Pseudaletia*) sp. GV #8 (MyspGV#8) from the Martignoni baculovirus collection, and also from occlusion bodies of a *Mythimna* (*Pseudaletia*) *unipuncta* NPV isolate MyunNPV-KY310 harvested from *M. unipuncta* in a

Kentucky, USA hayfield. Genomic DNA was subjected to 454 pyrosequencing, and the DNA sequences assembled using CLC Genomics Workbench and Lasergene SeqMan Pro software. The genomes for the three viruses ranged in size from 144,510 to 156,649 bp and contained all the core genes recognized for family *Baculoviridae*. Phylogenetic inference with concatenated amino acid sequence alignments of 34 core genes and pairwise comparisons of partial *lef-8*, *lef-9*, and *polh* nucleotide sequences revealed that all three of the *Mythimna* viruses represented currently unrecognized species of *Baculoviridae*, with MyspNPV#7 and MyunNPV-KY310 occurring in different locations in the *Alphabaculovirus* tree and MyspGV#8 occurring in the *Betabaculovirus* group. MyunNPV-KY310 was most closely related to LeseNPV, while MyspNPV#7 grouped in a clade with NPVs from *Mamestra* spp. MyspGV#8 grouped in a betabaculovirus clade containing PsunGV-H, but was distinct from PsunGV-H with Kimura-2-parameter nucleotide distances of 0.25 – 0.82 substitutions/site.

POSTER Wednesday 16:30 **VI-9**

Genome sequence of *Trichoplusia ni* granulovirus (TnGV), a novel sequenced betabaculovirus infecting the cabbage looper

Ma. de los Angeles Bivián-Hernández¹, Juventino López-Tlajomulco², Jorge E. Ibarra², Ma. Cristina Del Rincón-Castro¹

¹Food Department and Graduate Program in Biosciences, University of Guanajuato, Irapuato, GTO, México; ²Department of Biotechnology and Biochemistry, CINVESTAV-Irapuato, Irapuato, GTO, México

Correspondence: mdelrinc@yahoo.com

The cabbage looper, *Trichoplusia ni*, is a member of the moth family Noctuidae. It is an important pest in Canada, Mexico, and the United States. The cabbage looper feeds mainly on crucifers, and has been reported causing severe damage on broccoli, cabbage, cauliflower, Chinese cabbage, collards, kale, mustard, radish, rutabaga, turnip, and watercress, among others cultures. It is attacked by numerous natural enemies, including a nucleopolyhedrosis virus (NPV) and a granulovirus (GV). The latter is a *Betabaculovirus* isolated from the *T. ni* designated as TnGV, whose genome was sequenced in this work. The 175,360 pb long genome has a G+C content of 39.81% and a total of 178 ORFs coding for polypeptides of at least 50 amino acid residues. From the 178 ORFs, 16 showed early promoters, 13 of which were found in the positive and 3 in the negative strand; 63 ORFs showed late promoters, 43 in the positive strand and 33 in the negative one; 21 ORFs showed enhancer-like motifs, 12 in the positive strand and 9 in the negative one. The TnGV genome showed high synteny with the highly related genomes, such as those of *Pseudaletia unipuncta* GV, *Xestia c-nigrum* GV and *Helicoverpa armigera* GV. Interestingly, 11 ORFs were related to different NPV genes, three to *Heliothis virescens* ascovirus genes, and one related to a *Mythimna separata* entomopoxvirus gene. According to this comparison, the TnGV should be recognized as a new species from the genus *Betabaculovirus*, whose closest relative is *PsunGV* species, with 98.55% of identity.

POSTER Wednesday 16:30 **VI-10-STU**

The gene *pp31* of *Cydia pomonella* granulovirus is essential for the production of budded viruses and the establishment of a systemic infection in codling moth

Jacqueline A. Frank, Manuela Gebhardt, Diana Schneider, Jörg T. Wennmann, Johannes A. Jehle

Institute for Biological Control, Julius Kühn Institute (JKI), Federal Research Center on Cultivated Plants, Heinrichstraße 243, 64287 Darmstadt, Germany

Correspondence: Jacqueline.Frank@jki.bund.de

Cydia pomonella granulovirus (CpGV-M) (*Baculoviridae*, genus *Betabaculovirus*) is the most important commercial biological control agent of codling moth (CM) in orchards worldwide. However, only limited knowledge about the molecular basis of virus host interaction is available. We have initiated studies on CpGV genes, which may play a crucial role during the infection process. Transcriptional analysis revealed that gene *pp31* is an early and highly expressed gene in the midgut and fatbody of CM larvae. To examine the role of the *pp31* during CpGV infection, a knockout mutant was cloned using a gfp-tagged CpGV bacmid. After transfection of Cp14R cells neither production of budded viruses nor formation of occlusion bodies was observed for the *pp31* knockout mutant. This observation was confirmed by infection studies of CM larvae, which succumbed from infection from bacmid-based gfpCpGV but not from the knockout mutant. The influence of the deletion of *pp31* on DNA replication and transcription of different genes will be presented by qPCR. In contrast to nucleopolyhedroviruses, where deletion of *pp31* results in a reduction but not complete loss of the capacity of budded virus production, it is suggested for CpGV that *pp31* is an essential gene in the replication cycle.

POSTER Wednesday 16:30 **VI-11-STU**

Membrane binding and fusion play no role in cross-resistance against a granulovirus in a strain of *Adoxophyes honmai* selected for resistance to a nucleopolyhedrovirus

Kento Iwata, Maki N. Inoue, Yasuhisa Kunimi, Madoka Nakai
Tokyo University of Agriculture and Technology, Saiwai, Fuchu,
Tokyo 183-8509, Japan

Correspondence: madoka@cc.tuat.ac.jp

The smaller tea tortrix *Adoxophyes honmai* (Lepidoptera: Tortricidae) was selected for resistance to a nucleopolyhedrovirus (NPV) by repeatedly exposing a field-collected *A. honmai* NPV population to a 70% lethal concentration of *A. honmai* NPV (AdhoNPV) in the laboratory. The selected strain (resistant strain; R-strain) showed over 10,000-fold higher resistance to AdhoNPV than the non-selected strain (susceptible strain; S-strain). In previous work, using a fluorescence-dequenching assay to monitor membrane binding and fusion during primary infection, we showed that occlusion-derived viruses (ODVs) of AdhoNPV have lower binding and fusion ability with midgut epithelial cells of the R-strain than with those of the S-strain. Interestingly, the R-strain is cross-resistant to *A. orana* granulovirus (AdorGV), displaying an 846-fold higher resistance than the S-strain, although the mechanism is unknown. In this study, we conducted fluorescence-dequenching assays to determine whether cross-resistance of the R-strain against AdorGV is also attributable to a reduced affinity between AdorGV ODVs and R-strain midgut epithelial cells. The results showed that the binding and fusion ability of AdorGV ODVs to R-strain midgut epithelial cells is the same as it is to S-strain cells. These findings reveal that the cross-resistance against AdorGV does not involve binding and fusion of ODVs with midgut epithelial cells, but is based on some other mechanism.

POSTER Wednesday 16:30 **VI-12**

Enhancins and chitinases: Analysis of a granulovirus of *Spodoptera frugiperda*

Paola E. Cuartas¹, Emiliano Barreto², Gloria P. Barrera¹, Laura F. Villamizar¹

¹Corporación Colombiana de Investigación Agropecuaria, Corpoica, Bogotá, Colombia; ²Centro de Bioinformática, Instituto

de Biotecnología-IBUN, Universidad Nacional de Colombia, Bogotá, Colombia

Correspondence: pcuartas@corpoica.org.co

Baculoviruses are a large group of viruses pathogenic to arthropods, principally insect from the order Lepidoptera some of which are pests of agricultural importance. The main two genera of these viruses (Alphabaculovirus and betabaculovirus) have been used worldwide in biological control strategies. Related genes with virulence as *enhancins* and *chitinases* have been found in baculovirus, mainly in betabaculovirus. *Spodoptera frugiperda* granulovirus (SfGV) isolate possess two genes encoding to enhancins (ORFs 127 and 132) and four genes encoding to chitinases (ORFs 010, 071, 072 and 134) in its genome, being the betabaculovirus that would express more proteins associated to virulence. The aim of this work was to compare *enhancins* and *chitinases* of SfGV with homologous sequences described for other isolates of the family *Baculoviridae*. Bioinformatic analysis was performed with the deduced amino acid sequences for each gene. The enhancins of SfGV was grouped in phylogenetic cluster of betabaculovirus. The ORF 127 was classified in VEF-2 (viral enhancing factor) and grouped with *Helicoverpa armigera* granulovirus VEF-2 and *Xestia c-nigrum* betabaculovirus VEF-2, and the ORF 132 was classified as VEF-4 grouped with *Pseudaletia unipuncta* betabaculovirus VEF-4, *H. armigera* betabaculovirus VEF-4 and *X. c-nigrum* betabaculovirus VEF-4 (bootstrap values=100). All chitinases of SfGV lacked of C-terminal domain KDEL, present in most of baculovirus chitinases. The presence of this domain is related with lytic degradation of the integument of the larvae at the end of the infectious cycle; therefore the SfGV chitinases could be proteins secreted, associated with occlusion bodies and with proteolytic action at the initial stage of viral infection.

POSTER Wednesday 16:30 **VI-13**

Heterologous recombination between Baculoviruses: Horizontal transfer genes analysis

Gloria P. Barrera¹, Mariano N. Belaich², Paola Cuartas¹, Laura Villamizar², Pablo D. Ghiringhelli²

¹Corporación Colombiana de Investigación Agropecuaria, Corpoica, Bogotá, Colombia; ²Laboratorio de Ingeniería Genética y Biología celular y Molecular, Área Virosis de Insectos (LIGBCM-AVI), Dto. De Ciencia y Tecnología, Universidad Nacional de Quilmes, Provincia de Buenos Aires, Argentina

Correspondence: gbarrera@corpoica.org.co

Spodoptera frugiperda (Lepidoptera: Noctuidae) is an important pest for agriculture in America and it is a host for baculoviruses, both nucleopolyhedrovirus (NPVs) (Group II alphabaculovirus) and granulovirus (GVs) (betabaculovirus). In the present work detailed analysis of complete sequence (acquired by pyrosequencing) of a Colombian *S. frugiperda* granulovirus (SfGV) isolate was performed in order to explore processes of gene acquisition by horizontal transfer. The sequence analysis suggests events of gene acquisition by horizontal transfer including the ORFs 046/047 (paralogs), 059, 089 and 099. Three genes (ORF046, ORF047 and ORF089) are only shared with some betabaculoviruses and some alphabaculoviruses of Group II, while 2 other genes (ORF059 and ORF099) are shared only with alphabaculoviruses suggesting origins by horizontal transfer. Identity / similarity values of ORFs 046/047 respect to most similar proteins present in HearGV, PsunGV, XecnGV and SpltNPV II ranged between 27.4%-37.6% / 60.9%-63.3% which does not make clear the origin of this gene, however it could suggests that this relationship could be derived from an ancient acquisition and parallel evolution. SfGV ORF059 is only similar to the protein encoded in the ORF137 of SpltNPV II,

whereas SFGV ORF099 shows the highest identity / similarity values with ORF023 of the SfMNPV isolates (or ORF022 in SfMNPV 19). In both cases, the obtained results suggest the occurrence of independent horizontal transfer events during the evolution. Relative similarity and bootscanning analyses supported the occurrence of putative recombination events for SFGV ORF059 and ORF099 with high percentage of permuted trees by bootscanning plot against SfMNPVs and SpltNPV II suggesting a heterologous recombination events involving two different virus species belonging to two genera baculoviruses.

POSTER Wednesday 16:30 **VI-14**

Establishment of a winter moth, *Operophtera brumata*, cell line permissive for OpbrNPV replication

John P Burand¹; Robert L. Harrison², Matthew Boucher¹

¹Department of Microbiology, University of Massachusetts Amherst, MA, USA; ²USDA Agricultural Research Service, Beltsville, MD, USA

Correspondence: jburand@microbio.umass.edu

The winter moth, *Operophtera brumata*, is an invasive lepidopteran pest on deciduous tree species in the Northeast United States and Canada. We have recently found the previously identified nucleopolyhedrovirus OpbrNPV in *O. brumata* in Massachusetts and, as an initial step in developing this virus for the control of winter moth, we have established a cell line from this insect. Embryonated winter moth eggs were dissected, and pieces of embryo were transferred to TNM-FH and ExCell420 media supplemented with fetal bovine serum to establish primary cell cultures. Cultures were incubated at 19° C and sub-cultured using trypsin to produce an embryonic cell line, designated IIBBL-ObE1. The initial infection of this cell line was accomplished at passage 15 using occlusion-derived virus, and appearance of occlusion bodies (OBs) inside the nuclei of infected cells occurred with one week post infection. OBs recovered from infected cells were confirmed to be OpbrNPV by PCR and were infectious when fed to winter moth larvae.

POSTER Wednesday 16:30 **VI-15**

AcMNPV infection process in *Trichoplusia ni* midgut

Muhammad Afzal Javed¹, Stephanie Harris¹, David A. Theilmann², Martin A. Erlandson¹, Dwayne D. Hegedus¹

¹Saskatoon Research Centre, Agriculture and Agri-Food Canada, Saskatoon, SK, S7N 0X2 Canada; ²Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Summerland, BC, V0H 1Z0 Canada

Correspondence: Afzal.javed@agr.gc.ca

Baculoviruses infect a wide range of insect hosts, including important crop pests and as such are potentially excellent tools for biocontrol of these pests. *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) is the type species for the genus *Alphabaculovirus* of the family *Baculoviridae*. AcMNPV infection consists of two distinct phenotypes, budded viruses and occlusion derived viruses (ODVs). Previously, it has been shown that AcMNPV infection starts upon oral ingestion of occlusion bodies (OBs) by larvae. OBs dissolve in the alkaline environment of the lepidopteran larvae midgut to release ODVs that bind to and enter midgut epithelial cells. Foci of AcMNPV infection form in the midgut; however, it is not clear if these foci of infection form from primary infections or progeny virions spreading to neighboring midgut cells. We report details of AcMNPV infection in midgut tissue using AcMNPV viruses expressing reporter fluorescence proteins, GFP or VP39-3X-mCherry fusion proteins, and a *gp64* knockout virus. Oral infection of *Trichoplusia ni* larvae with GFP-

recombinant AcMNPV, surrogate for wild type virus, showed that the anterior part of midgut is more heavily infected compared to the posterior midgut. Initially, individual midgut cells are infected and subsequently infection spreads to neighboring cells in midgut epithelial layer. The spread of the infection from primary infected cells is dependent upon GP64. Tracheoles and hemocytes are subsequently infected at approximately 48 hour post infection. Midgut infection declines by 72 hours post infection.

POSTER/ Wednesday 16:30 **VI-16-STU**

The effects of heterologous p10 expression in *Autographa californica* multicapsid nucleopolyhedrovirus replication in insect cells

Leo Graves¹, Sarah L. Irons¹, Robert D Possee^{1,2}, Linda A King¹

¹Department of Biological and Medical Sciences, Oxford Brookes University, Oxford UK; ²Oxford Expression Technologies Ltd, Oxford, UK

Correspondence: 12083983@brookes.ac.uk

P10 is a small, highly expressed fibrous protein that forms a complex network of filaments and a distinct tubular structure (perinuclear cage) around the nucleus during the later stages of baculovirus infection. Possible functions of P10 are in nuclear stability, polyhedron formation and cell lysis, but distinct mechanisms to account for these roles have yet to be determined.

To investigate the species-specific role of P10 during infection an *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV) mutant was constructed, in which the AcMNPV p10 coding region was replaced with that from *Spodoptera frugiperda* (Sf)NPV. Replacement of this region with SfNPV p10 resulted in a virus, AcMNPV-P10^{Sf}, with decreased polyhedra formation, low budded virus (BV) titre and aberrant rearrangement of microtubules (MT). This suggested that SfNPV-P10 may be affecting translocation of nucleocapsids to the plasma membrane via MT for budding.

Using a Sfp10 specific antibody, Sfp10 expression was only weakly visible in AcMNPV-P10^{Sf} infected Sf cells in comparison to the SfNPV infection. To determine if the low BV titre and reduced polyhedra were a result of Sfp10 expression, siRNA was used to reduce P10 synthesis, which resulted in a significant increase in both polyhedrin expression and polyhedra formation. There was also a partial increase in BV titre. These data suggested that P10 has clear species specific roles.

POSTER Wednesday 16:30 **VI-17-STU**

Multiple amino acid residues of *Autographa californica* MNPV P143 are responsible for ribosomal RNA degradation in *Bombyx mori* cells

Rina Hamajima, Michihiro Kobayashi, Motoko Ikeda

Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya 464-8601, Japan

Correspondence: q2u_q1@yahoo.co.jp

BM-N cells derived from the silkworm, *Bombyx mori*, are permissive for homologous *B. mori* NPV (BmNPV), while non-permissive for *Autographa californica* multiple NPV (AcMNPV). We previously showed that the ribosomal RNA (rRNA) degradation is induced in AcMNPV-infected BM-N cells, and triggered by P143 of AcMNPV (Ac-P143).

In this study, we analyzed Ac-P143 to identify the amino acid residues responsible for rRNA degradation. P143 is a DNA helicase essential for viral DNA replication. To determine whether DNA helicase activity of P143 is involved in the rRNA degradation, we

examined rRNA of BM-N cells infected with AcMNPV temperature sensitive mutant 8 (ts8) defective in DNA helicase activity of P143 at non-permissive temperature (33°C). The result showed that rRNA degradation was induced at both permissive and non-permissive temperature, indicating that DNA helicase activity of P143 is not associated with rRNA degradation in AcMNPV-infected BM-N cells. Next, we constructed mutated Ac-P143s containing amino acid residue substitutions with corresponding those of BmNPV P143 that does not trigger the rRNA degradation, and analyzed using transient expression assay. The results demonstrated that six to eight amino acid residues of Ac-P143 located within the region related to restriction of NPV replication in *B. mori* cells are responsible for the rRNA degradation of BM-N cells. Our analysis using single cell based assay further demonstrated that eight amino acid residues are also involved in restriction of NPV replication in BM-N cells.

POSTER Wednesday 16:30 **VI-18**

***Autographa californica* multiple nucleopolyhedrovirus ORF11 and ORF78 are essential for budded virus production, occlusion-derived virus envelopment, and occlusion body formation**

Xue Ying Tao^{1,2}, Woo Jin Kim³, Jae Young Choi⁴, Seok Hee Lee³, Jong Hoon Kim³, Pang Ying³, Ha Kyu Baik³, Yeon Ho Je³

¹State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang, China; ²Jiangxi-OAI Joint Research Institute, Nanchang University, Nanchang, China; ³College of Agriculture and Life Science, Seoul National University, Seoul, Republic of Korea; ⁴Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul, Republic of Korea

Correspondence: woojin.kim@snu.ac.kr

ORF11 and ORF78 of *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) are highly conserved genes of unknown function. To determine the role of these genes in baculovirus replication cycle, two AcMNPV mutants, Ac11KO and Ac78KO with *ac11* and *ac78* deleted, were constructed, respectively. Microscopy, titration assays, and Western blot analysis revealed that budded viruses (BVs) were not produced in Ac11KO-transfected Sf9 cells. However, qPCR analysis demonstrated that the deletion of *ac11* did not affect viral DNA replication. Electron microscopy showed that there was no nucleocapsid in the cytoplasm or plasma membrane of Ac11KO-transfected cells, which demonstrates that the defect in BV production in Ac11KO-transfected cells is due to the inefficient egress of nucleocapsids from the nucleus to the cytoplasm. Also it was observed that the nucleocapsids in the nucleus were not enveloped to form occlusion-derived viruses (ODVs) and their subsequent embedding into occlusion bodies (OBs) was also blocked in Ac11KO-transfected cells. These results demonstrate that *ac11* is essential for BV production and ODV envelopment. Ac78KO-transfected cells produced a single-cell infection phenotype, indicating that no infectious BVs were produced. The defect in BV production was also confirmed by both viral titration and Western blotting. However, viral DNA replication was unaffected, and OBs were formed. An analysis of BVs and ODVs revealed that AC78 is associated with both forms of the virions and is an envelope structural protein, and also plays an important role in the embedding of ODV into the OB, which is essential for the viral life cycle.

POSTER Wednesday 16:30 **VI-19-STU**

Effects of lacking non-essential genes of BmNPV

Hitomi Taka¹, Chikako Ono², Masanao Sato³, Shin-ichiro Asano¹, Hisanori Bando¹

¹Graduate School of Agriculture, Hokkaido University, Sapporo, Japan; ²Research Institute for Microbial Diseases, Osaka University, Suita, Japan; ³Institute for Advanced Biosciences, Keio University, Tsuruoka, Japan

Address for Correspondence: hban@abs.agr.hokudai.ac.jp

Nucleopolyhedroviruses (NPV, a member of the family Baculoviridae) produces copious amounts of polyhedrin protein by the end of a replication cycle forming a lot of polyhedra in the nuclei of infected-host insect cells. This characteristic feature of NPV is a beneficial trait as the gene expression vector. To further develop baculoviral applications, deep insight into the functions and interactions of viral gene products concerning the explosive expression of Polh is necessary.

We constructed a comprehensive knockout virus library of BmNPV T3 and showed 86 genes out of 141 BmNPV T3 genes were dispensable for expression of the polyhedrin gene and production of infectious viral progenies (Ono et al., 2012). In BmNPV genome, there were 10 regions containing only non-essential genes (non-essential gene clusters, NEGCs). In order to analyze the effects when these clusters were deleted, we constructed BmNPV bacmids lacking each NEGC and analyzed the expression of GFP under the control of the polyhedrin gene promoter.

Out of ten BmNPV bacmids lacking one of the NEGCs, only one bacmid lacking bm32-bm38 cluster was deficient in production of GFP and/or infectious progeny viruses in transfected cells. This result showed some combinations of non-essential genes have important role for expressing Polh. In addition, we constructed BmNPV bacmids lacking the 7 non-essential genes (bm32-bm38) in various combinations. We report here results from phenotypic analysis of these multiple-gene knockout bacmids.

POSTER Wednesday 16:30 **VI-20-STU**

Relevance of BmSyndecan-1 for BmNPV proliferation

Momoka Uda¹, Masanao Sato², Shin-ichiro Asano¹, Hisanori Bando¹

¹Graduate School of Agriculture, Hokkaido University, Sapporo, Japan; ²Institute for Advanced Biosciences Keio University, Yamagata, Japan

Correspondence: m-uda@abs.agr.hokudai.ac.jp

Baculovirus is a well-known natural pathogen to insects. It is a well-studied virus but not much is known about the entry mechanisms particularly secondary infection and attachment to general cell surface molecule via non-specific electrostatic interaction is most probable. Baculoviruses are insect specific virus and cannot replicate in mammalian cells. However *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV), belonging to genus Alphabaculovirus, can enter the mammalian cells. Recently it was reported that AcMNPV utilized part of heparin sulfate chain of Syndecan-1 when entering the mammalian cells (Makkonen et al., 2014). This was the first report that baculovirus may utilize some specific molecule as their receptor. At present we have no information if the mechanism of baculovirus entering mammalian cells applies to the cases in insect cells. We here studied this topic in the infection of Bombyx mori nucleopolyhedrovirus (BmNPV) to BmN cells of silkworm. Syndecan-like transcript variant-1 (BmSyndecan-1) and Syndecan-like transcript variant-2 (BmSyndecan-2) have been detected in the silkworm. First we investigated whether BmSyndecan-1 was required in BmNPV proliferation in BmN cells using RNAi technology to knock down the objective gene. So far the knocking down of BmSyndecan-1 showed no significant effect on virus proliferation in BmN cells. We are now conducting RNAi targeting other molecules i.e. BmSyndecan-2.

POSTER Wednesday 16:30 **VI-21-STU****Applications of DnaB mini-intein to baculovirus expression system**Won Seok Gwak, Sung Min Bae, Tae Young Shin, Seung Hee Lee, Yong Oh Ahn, Soo Dong Woo

Department of Agricultural Biology, College of Agriculture, Life & Environment Science, Chungbuk National University, Cheongju 361-763, Korea

Correspondence: sdwoo@cbnu.ac.kr

Recombinant proteins including a polypeptide fusion partner, termed affinity tag, to facilitate the purification of the target polypeptides are widely used. When designing up- and downstream processing strategy for a protein, the inclusion of an affinity tag might be attractive for a number of additional reasons. However, the disadvantage of affinity tags is that, in many studies, the tag must be removed from the target after the purification process. Recently, the *Synechocystis* sp. PCC6803 DnaB mini-intein (Ssp DnaB mini-intein) is widely used in *Escherichia coli* expression systems as the solution of this problem. The Ssp DnaB mini-intein can be induced simply by shifting of pH and temperature, offering a benefit to cleave a peptide bond without using a protease or chemical reagent. Although the utility of this novel tag is widely studied in *E. coli*, there is no report yet in baculovirus expression vector system (BEVS). In this study, we generated several recombinant baculoviruses to express foreign proteins with Ssp DnaB mini-intein. The optimal conditions for the Ssp DnaB mini-intein in BEVS and the characteristics of recombinant proteins having Ssp DnaB mini-intein were analyzed. In conclusion Ssp DnaB mini-intein was good tag also in BEVS with more advantages.

POSTER Wednesday 16:30 **VI-22-STU****Production of porcine parvovirus virus-like particles using baculovirus in the silkworm larvae**Seung Hee Lee, Sung Min Bae, Tae Young Shin, Won Seok Gwak, Yong Oh Ahn, Soo Dong Woo

Department of Agricultural Biology, College of Agriculture, Life & Environment Science, Chungbuk National University, Cheongju 361-763, Korea

Correspondence: sdwoo@cbnu.ac.kr

Porcine parvovirus (PPV), a member of the genus Parvovirus, family Parvoviridae, is a significant causative agent in porcine reproductive failure, causing serious economic losses in the swine industry. We have studied usefulness of recombinant porcine parvovirus virus-like particles (PPV-VLPs) as an efficient recombinant vaccine. PPV is a non-enveloped virus and its capsid is assembled from three viral proteins (VP1, VP2, and VP3). The major capsid protein, VP2 is the main target protein for neutralizing antibodies in PPV. When VP2 was expressed in large amounts, it assembled into virus-like particles (VLPs) similar in size and morphology to the original virions. In this study, we generated the recombinant *Bombyx mori* nucleopolyhedrovirus (BmNPV) to express the VP2 protein. Expression of the VP2 protein was analyzed by SDS-PAGE and Western blot approximately 64 kDa in insect cells and larvae. The formation of VLP by recombinant VP2 was confirmed through transmission electron microscopy examination that diameters ranging from 20 to 22 nm. Additionally, Classical Swine Fever Virus (CSFV) E2 epitope sequences were inserted into the VP2 capsid protein each of N-term, C-term or both sides [PPV-VLP(CSFV)] to expand the utility of PPV-VLP as a vaccine. Production of the PPV-VLP(CSFV) was analyzed by approximately 65 kDa and also identified by transmission electron microscopy.

POSTER Wednesday 16:30 **VI-23****Transcriptional analysis of the putative glycosyl transferase gene (amv248) of *Amsacta moorei* entomopoxvirus**Cihan Inan¹, Hacer Muratoglu², Basil Arif³, Zihni Demirbag¹¹Department of Biology, Faculty of Sciences, Karadeniz Technical University, Trabzon, Turkey; ²Department of Molecular Biology and Genetics, Faculty of Sciences, Karadeniz Technical University, Trabzon, Turkey; ³Laboratory for Molecular Virology, Great Lakes Forestry Centre, Sault Ste. Marie, Ontario, CanadaCorrespondence: zihni@ktu.edu.tr

Amsacta moorei entomopoxvirus (AMEV), the most studied member of Betaentomopoxvirus genus, isolated from the larvae of the *Amsacta moorei* moth and distant relative of the better studied orthopoxviruses including variola and vaccinia virus. The virus genome consists of 294 open reading frames (ORF) in which AMV248 is known to encode a putative glycosyl transferase protein. AMV248 includes 870 nucleotides that encodes 279 amino acids and has a transmembrane domain. Studies of AMV248 orthologs showed that the protein is a member of entry fusion complex and responsible for viral attachment to the host cell. RT-PCR analysis of virus specific RNAs indicated that the transcription of the gene started between 3-6 h post-infection (hpi) and continued to be expressed through 24 hpi. While infection of AMEV in *Lymantria dispar* (Ld652) cells occurs in the presence of Ara-C, it is inhibited in the presence of cycloheximide (CHX). It shows that AMV248 is transcribed as an intermediate gene of temporally expressed viral transcripts. 5' RACE analysis of AMV248 showed that transcription starts at 126 bp upstream region which is 204704th base in AMEV full genome. 3' RACE analysis shows that 3' untranslated region of AMV248 is probably between 50th and 84th bases after stop codon. This study was supported financially by The Scientific and Technological Research Council of Turkey (TUBITAK, Project No. 113Z219).

POSTER Wednesday 16:30 **VI-24-STU****CrV1 mimics host α -tubulin to sequester GAPDH, which plays a crucial role in cytoskeleton rearrangement**Kumar Sunil, Yonggyun Kim

Department of Bioresource Sciences, Andong National University, Andong 760-749, Republic of Korea

Correspondence: sunilkumar_micro@rediffmail.com

Insect immunity is innate and consists of cellular and humoral immune responses. Cellular response usually requires hemocyte spreading behavior, which is accompanied by cytoskeletal rearrangement. A glycolytic enzyme, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), catalyzes an oxidation reaction of glyceraldehyde-3-phosphate to change in to 1, 3-bisphosphoglycerate in the cytosol. Another function of GAPDH in mammalian cell is to bind C-terminal of α -tubulin to facilitate cytoskeletal arrangement. An immunoprecipitation of viral protein, CpBV-CrV1, against hemocyte lysate revealed that CpBV-CrV1 binds to GAPDH, identified by MALDI-TOF analysis. dsRNA specific to GAPDH inhibits hemocyte spreading function, while RNAi treatments of other glycolytic genes had no effect on spreading. The common molecular motif of CpBV-CrV1 and α -tubulin at C-terminal supported the IP analysis. To test the role of α -tubulin motif in CpBV-CrV1, point mutations of CpBV-CrV1 were applied that's result in loss of the biological activity of CpBV-CrV1. Furthermore immunofluorescence assay indicates CpBV-CrV1 colocalized with tubulin in hemocytes collected from *Plutella xylostella* parasitized by *cotesia plutellae*. This result suggests that GAPDH plays a critical role in hemocyte spreading behavior during

immune challenge, and it is a molecular target of a pathogenic virus.

POSTER Wednesday 16:30 **VI-25**

The use of zinc-based fixatives for high-fidelity histomorphology and molecular histochemical techniques on arthropod tissue

Damien M. Laudier

Laudier Histology, New York City, NY

Correspondence: dmlaud@laudierhistology.com

Producing good histological preparations of arthropod tissues is often problematic. Studies that require high-fidelity morphological preservation for histomorphometric measurements, qualitative/quantitative histopathology and spatial localization of histochemical events; inconsistent or poor histologic preparations can render samples useless for light microscopy analysis. The underlying causes of this variability are not always conclusive. The choice of a tissue fixative is a significant factor in determining the quality outcome of an arthropod histologic preparation. Zinc-based fixatives have proven to provide exceptional morphological preservation, retention of DNA/RNA integrity and antigenic properties of vertebrate tissue. However, the application of zinc-based fixatives on arthropod tissues has been limited. Focusing on class Insecta and class Arachnida, this study compares histological preparations of select tissue types such as neuronal, ocular, digestive and reproductive. Specimen samples were fixed immediately post-sacrifice with various zinc-based fixatives, or with commonly used formaldehyde-based fixatives (zinc-free) for comparison. Following fixation, all tissue samples were comparably processed, embedded and sectioned. Staining was either tinctorial or with an antigenic marker identifying a particular protein expression via immunohistochemical technique. The results illustrate that zinc-based fixatives are highly effective in maintaining high-quality tissue integrity for simple and complex histological investigations and are empirically superior to conventional fixatives.

THURSDAY – August 13th

NEMATODE SYMPOSIUM Thursday 8:00 – 10:00

Recent Advances in Entomopathogenic Nematode Infection Behavior: Inside and Outside

SYMPOSIUM PAPER Thursday 8:00 **125**

Advances in entomopathogenic nematode dispersal and host-finding behavior

David Shapiro-Ilan¹; Edwin E. Lewis²

¹USDA-ARS, SEFTNRL, Byron, GA, USA; ²Department of Entomology and Nematology, University of California, Davis, USA

Correspondence: David.Shapiro@ars.usda.gov

Successful infection by entomopathogenic nematodes (epns) depends on being able to disperse and find the host. Therefore, to expand our understanding of epn ecology and leverage their use in biocontrol, it is imperative that we elucidate the behavioral mechanisms behind EPN movement and host-finding. Some recent findings and topics that will be discussed include enhanced dispersal from nematode infected hosts, the discovery of novel cues that induce epn movement such as electromagnetic signals

(Ilan et al. 2013), and group movement behavior (moving together in pack) (Shapiro-Ilan et al. 2014).

Ilan, T., D.B. Kim-Shapiro, C. Bock, and D.I Shapiro-Ilan. 2013. The impact of magnetic fields, electric fields and current on the directional movement of *Steinernema carpocapsae*. International Journal of Parasitology, 43: 781-784.

Shapiro-Ilan, D.I, E.E. Lewis, and P. Schliekelman 2014. Aggregative group behavior in insect parasitic nematode dispersal. International Journal of Parasitology 44: 49-54.

SYMPOSIUM PAPER Thursday 8:30 **126**

Sex, age and following the leader drive infection dynamics of entomopathogenic nematodes

Ed Lewis¹, David Shapiro-Ilan², Yohandra Gonzales¹, Danica Maxwell¹

¹Department of Entomology and Nematology, University of California – Davis, Davis, CA, USA; ²USDA-ARS, SEFTNRL, Byron, GA, USA

Correspondence: eelewis@ucdavis.edu

Entomopathogenic nematodes (EPNs) employ a mass-attack strategy when they infect a host. This strategy is favored because, in most cases, a single infective juvenile (IJ) is unable to overcome host defenses, and for EPNs in the genus *Steinernema*, females and males are needed for reproduction. It is likely that EPNs move in groups to facilitate this sort of strategy. But, even in a mass attack, one individual must be first to invade, and others must follow. Three questions are addressed here. (1) Which individuals invade first? (2) How many individuals follow? and (3) How long does the infection window stay open? Behavioral differences among individual IJs have been documented numerous times, and can be used to address these questions. Males and females differ in responses to hosts; males have been dubbed the colonizing sex. Old IJs respond differently to hosts than do young IJs; risk-sensitive foraging theory suggests that IJs with less lifespan remaining might take more risks than younger IJs. The interplay between individuals' behavior and group dynamics is our focus in this work.

SYMPOSIUM PAPER Thursday 9:00 **127**

Impact of infection behaviour on lethal male fighting in *Steinernema*

Apostolos Kapranas, Abigail M. D. Maher, Annemie Zenner, Kate O'Callaghan, Christine T. Griffin

Biology Department, Maynooth University, Maynooth, County Kildare, Ireland

Correspondence: christine.griffin@nuim.ie

Males of certain species of *Steinernema* fight and kill each. The combat takes place within the killed host insect, a valuable resource that was previously colonised by infective juveniles (IJs) and killed with the aid of their bacterial symbiont. The host cadaver is thus a closed system in which the number and relatedness of competitors for adult male nematodes is determined by the infection behaviour of the IJs. The recent discovery of lethal fighting in these entomopathogenic nematodes provides a useful invertebrate model for testing theoretical predictions regarding factors such as group size and relatedness of individuals on the outcome of fighting. According to the theory of kin selection, individuals should show less aggression towards close relatives. We tested the effect of relatedness on aggression in groups of *S. longicaudum*, using nine inbred lines, and examined how competitor male group size and relatedness influence male mortality rates. In a series of *in vitro* experiments we found that both relatedness and competitor male group size influenced male mortality rates. Higher relatedness led to

progressively lower rates of male mortality. These *in vitro* findings were supported by results of experiments in insects. The results collectively show that male mortality rates are lower in groups consisting of more related males, indicating that male entomopathogenic nematodes recognise their kin. We discuss the results in the context of the underlying population structure of entomopathogenic nematodes and their dispersal and infection behaviour.

SYMPOSIUM PAPER Thursday 9:30 **128**

The stability of virulence in insect parasitic nematodes is determined by social interactions

Ben Raymond¹, David Shapiro-Ilan²

¹Imperial College London, Silwood Park campus, Ascot, Berks, SL5 7PY, UK. ²USDA-ARS, South Eastern Fruit and Nut Research, Byron, USA

Correspondence: b.raymond@imperial.ac.uk

Cooperative secretion of virulence factors by pathogens can often lead to social conflict as cheating mutants that benefit from collective action, but do not contribute to it, can arise and locally outcompete cooperators within hosts. Since cooperation is important for the virulence of invertebrate pathogens such as *Bacillus thuringiensis* social biology might be applied to the maintenance and improvement of biocontrol agents. Entomopathogenic nematodes (EPN) are ideal candidates here: EPN depend partly on symbiotic bacteria to infect and kill hosts and both bacteria and nematode secrete diverse virulence factors. Critically, effective EPN strains are hard to maintain without serial propagation *in vivo* (passage), and this can lead to attenuation. We tested whether cooperation is important for the maintenance of virulence in the EPN *Heterorhabditis floridensis*. As predicted, the potential for social conflict dramatically affected EPNs; low relatedness (high multiplicity of infection), which is expected to increase the success of cheats, led to reduced virulence, lower reproductive rates and premature extinction of all replicates in that treatment within 7 passages. Cooperation also requires strong global (between-host) competition. Contrary to expectation, our attempt to increase between-host competition led to reduced virulence. We conclude that it was difficult to manipulate between-host competition without affecting relatedness. Importantly, passage treatments that maintained high relatedness led to stable virulence and increased nematode reproductive rates, suggesting selection experiments aimed at nematode improvement might benefit from taking social interactions into account.

CONTRIBUTED PAPERS Thursday 8:00 – 10:00

Fungi 2

CONTRIBUTED PAPER Thursday 8:00 **129-STU**

Transcriptomic and physiological responses of *Arabidopsis thaliana* to endophytic *Beauveria bassiana*

Maya Raad, Travis Glare, Michael Rostás

Bio-Protection Research Centre, PO Box 85084, Lincoln University, Lincoln 7647, New Zealand
maya.raad@lincolnuni.ac.nz

While the direct pathogenic activity of *Beauveria bassiana* against insects is well characterized, indirect fungus-insect interactions mediated by the host plant have been little studied. Information on how plants respond to the biocontrol agent is needed to obtain a comprehensive picture of the molecular basis of this

interaction. Microarray analysis was performed with *B. bassiana* growing endophytically in *Arabidopsis thaliana*. The comparative transcriptome study revealed 1015 differentially expressed genes (DEG). The majority (66%) of DEG were down-regulated, while 34% were up-regulated. Gene ontology classification demonstrated that many DEG were involved in responses to abiotic and biotic stimuli and in developmental processes and protein metabolism. MapMan analysis showed that large numbers of genes related to cell wall activity were down-regulated. Others related to abiotic stress were up-regulated. Genes involved in defence regulation such as transcription factors (WRKY, ERF) and hormones signalling (ethylene, auxin, and salicylic acid) were down-regulated. This correlated with the finding that jasmonic and salicylic acid levels were neither induced nor primed by *B. bassiana* and confirmed the absence of its antagonistic effects on two herbivore species. In conclusion, plant responses to *B. bassiana* rather resemble those to a symbiotic endophyte (e.g. *Trichoderma* spp.) than to a pathogen.

CONTRIBUTED PAPER Thursday 8:15 **130**

Non-entomopathogenic role of entomopathogenic fungi in strawberry production

Surendra K. Dara

University of California Cooperative Extension, San Luis Obispo, USA

skdara@ucdavis.edu

In light of recent studies in strawberries and cabbage in California that demonstrated the role of *Beauveria bassiana* and *Metarhizium brunneum* in promoting plant growth and health through endophytic and mycorrhizal interactions, a field study is currently underway in commercial strawberry field to further investigate their role. Impact of *B. bassiana*, *M. brunneum*, and *Isaria fumosorosea* on the growth, plant health, disease incidence, and yield of strawberries is evaluated. Various commercial products, including those based on beneficial microbes, are also compared in the study. Preliminary data show a positive impact of some of the fungi on some of the parameters measured. Non-entomopathogenic role of entomopathogenic fungi can enhance their appeal and use in agriculture.

CONTRIBUTED PAPER Thursday 8:30 **131-STU**

Phenotypic diversity among isolates of the entomopathogenic endophyte *Beauveria bassiana* affects plant-host interactions

Aimee McKinnon¹, Travis R. Glare², Hayley Ridgway², Andrew Holyoake², Artemio Mendoza Mendoza¹

¹Bio-Protection Research Centre, Lincoln University, Christchurch, New Zealand; ²Faculty of Agriculture and Life Sciences, Lincoln University, Christchurch, New Zealand

Correspondence: aimee.mckinnon@lincolnuni.ac.nz

Isolate selection from species of *Beauveria* utilised for biocontrol is generally based on a range of characteristics such as conidiation, metabolite production and virulence to the host targeted. This is because, the variation among closely related isolates within the species can range significantly, even under similar conditions. It is reasonable, therefore, to hypothesise that isolate variation may also occur for the interaction with a specific plant when assessing endophytic, epiphytic and rhizosphere colonisation. Furthermore, in order to ascertain the genotypic basis for live plant tissue interaction within the *B. bassiana* species and characterise the suitability of these interactions for potential plant protection, reliable phenotypic differences must first be identified between isolates. Experiments were conducted under controlled conditions to compare three isolates of *B.*

bassiana in association with sweetcorn (*Zea mays*). The first set of experiments focused on plant growth effects, using the invasive inoculation method developed for endophytes (*Epichloë*) of perennial ryegrass. The second set assessed rhizosphere ecology over time, using a root-dip inoculation method and included a herbivory simulation treatment. The quantity of *Beauveria* in the rhizosphere versus the bulk soil, and in association with plant material was compared between isolates using soil dilution plating techniques and an optimised PCR-based *Beauveria* detection method. Additionally, the microbial community structure and function was assessed using a DGGE and MicroResp™ analysis. Significant differences between the isolates studied were observed during various plant-host interactions, suggesting that phenotypic diversity may play a role in the plant associated life history strategy, with important ecological implications.

CONTRIBUTED PAPER Thursday 8:45 **132**

Light affect sporulation patterns of the mite pathogenic fungus *Neozygites floridana*

*Ingeborg Klingen*¹, *Maren Pindslø Holthe*^{1,2}, *Arupillai Suthaparan*², *Karin Westrum*¹, *Torfinn Torp*¹

¹Norwegian Institute for Agricultural and Environmental Research (Bioforsk), Norway, ²Norwegian University of Life Sciences, Department of Plant Sciences, Norway
Correspondence: ingeborg.klingen@bioforsk.no

A controlled microcosm experiment was conducted to examine how light affects the sporulation pattern of the mite pathogenic fungus *Neozygites floridana* during a 24 h cycle over a period of eight days. This was done by inoculating two spotted spider mites (*Tetranychus urticae*) with *N. floridana* and placing them on strawberry plants for death and sporulation. Spore discharge was observed by using a spore trap. Two light regimes were tested: Plant growth light of 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for 12 h supplied by high pressure sodium lamps (HPS), followed by either; i) 4 h of 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light with similar lamps followed by 8 h darkness or ii) 4 h of 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ red light followed by 8 h darkness. The red light treatment resulted in a greater number of spores over a longer period of time than the light supplied by HPS. A clear difference in hourly spore discharge pattern between the two different light treatments was seen and a significant interaction effect between light treatment and hour in day during the 24 h cycle was observed. The spore discharge peak for the red light treatment is reached within the red light hours and the dark hours. The spore discharge peak for the light supplied by HPS lamps is reached within the dark hours. The results suggest that light affects the sporulation of the beneficial fungus *N. floridana* indirectly through multitrophic interactions between the plant (strawberry), the herbivore (*T. urticae*) and the beneficial (*N. floridana*).

CONTRIBUTED PAPER Thursday 9:00 **133**

Environmental safety of Canadian *Metarhizium* S54 for the northern crayfish, *Orconectes virilis*, and phantom midge larva, *Chaoborus americanus*

*Dan Johnson*¹, *Larry Kawchuk*², *Stefan Jaronski*³, *Craig Wiebe*¹, *Zhe Zhang*¹

¹Water and Environmental Science Building, University of Lethbridge, Lethbridge, Alberta, Canada T1K 3M4; ²Research Centre, Agriculture and Agri-Food Canada, Lethbridge, Alberta, Canada T1J 4B1; ³Pest Management Research Unit, Northern Plains Agricultural Research Laboratory, USDA Agricultural Research Service, Sidney, Montana, USA 59270
Correspondence: dan.johnson@uleth.ca

The entomopathogenic fungus *Metarhizium anisopliae*, Canadian isolate S54, is being developed as a control agent for use to reduce damage caused by grasshoppers (Orthoptera: Acrididae), and other agricultural and urban insects. Candidate biopesticides must be safe for aquatic environments, because of possible runoff or overspray. The objective of this study was to assess the effects of this biopesticide candidate on two non-target aquatic invertebrates: the northern crayfish *Orconectes virilis* and the phantom midge larva *Chaoborus americanus*. S54 cultures were originally isolated from soil in Alberta, and cultured to produce dry conidia. Healthy adult crayfish (*O. virilis*) were collected and housed in 28 aquaria. Entomopathogen exposure experiments were applied to water in the tanks at two exposure rates, plus controls, for two 21-day intervals. In a separate experiment, larvae of the midge *Chaoborus americanus* (an ecologically important prey item for fish) were exposed to spore concentrations or chemical insecticides, in 42 1-L glass containers, and mortality was assessed. Treatment with S54 did not result in observed changes in mortality, feeding, movement, or aggression, in the crayfish over the course of 42 days. Dissections and microscopic examination of crayfish indicated no growing hyphae. S54 did not result in short-term (5 days) or long-term (assessed up to 30 days) mortality of *C. americanus*. The three insecticide treatments resulted in 100% mortality of *C. americanus* by day 5. We conclude that use of *Metarhizium* S54 for insect pest control is unlikely to pose a hazard to these prominent aquatic invertebrates.

CONTRIBUTED PAPER Thursday 9:15 **134**

Temporal density dependence of *Entomophaga maimaiga*

Ann E. Hajek

Department of Entomology, Cornell University, Ithaca, USA
Correspondence: ae4@cornell.edu

Sampling has been conducted at a site in New York State over 18 years to follow the *Lymantria dispar* population and its associated pathogens and parasitoids. The dominant pathogen was *Entomophaga maimaiga*, which was temporally density dependent and its prevalence was also associated with rainfall. *Lymantria dispar* nucleopolyhedrovirus infections were only very abundant during the early years of the study, when *L. dispar* populations were at higher population densities and *E. maimaiga* was just moving into this area. Four species of parasitoids were present throughout the years but rarely co-infected with *E. maimaiga*. The literature reports cyclicity (5 or 10 year) in *L. dispar* population in the more southerly mid-Atlantic region, but at this site, no cycles occurred; after an initial population collapse, the *L. dispar* population did not increase to densities causing any defoliation over the 18 years of this study.

CONTRIBUTED PAPER Thursday 9:30 **135**

Importance of mechano-signal for fungus removal in *Drosophila* grooming

*Aya Yanaqawa*¹, *Toshimitsu Hata*¹, *Tsuyoshi Yoshimura*¹, *Frederic Marion-Poll*^{2,3}

¹Kyoto University, Uji, Japan; ²CNRS, Laboratoire Evolution, Génomes, Comportement et Ecologie, Gif-sur-Yvette, France; ³AgroParisTech, Département Sciences de la Vie et Santé, Paris, France

Correspondence: ayanagawa@rish.kyoto-u.ac.jp

Insects remove and clean microbes from by their surface grooming themselves, which is considered as a behavioral defense against pathogen/parasite infection in some cases. Insects like *Drosophila melanogaster*, which live in an environment littered

with bacteria, fungi and other microorganisms developing on decaying material devote a lot of time to self-grooming which seems to contribute cleaning their cuticula from external particles. The sensory cues that trigger this behavior are still ambiguous. Previously, we showed that *D. melanogaster* can sense chemical cues from bacteria that induce grooming following the activation of taste neurons. In this study, we examined if flies remove fungal conidia from their cuticula by grooming and if this behavior involves taste sensilla. To this mean, we compared grooming activities of flies normal flies with that of mutant flies (*poxn*), deprived of external taste sensilla. Normal and *poxn* flies were equally efficient in their removal of fungal conidia by grooming. This suggests that in *D. melanogaster*, fungal conidia are not detected by contact chemical stimuli, but rather through mechanical or odorant stimuli.

CONTRIBUTED PAPER Thursday 9:45 **136-STU**

Older beetles are stronger than young: Influence of mating and age on susceptibility to a fungal pathogen

Joanna J. Fisher, Ann E. Hajek

Department of Entomology, Cornell University, Ithaca, USA

Address for Correspondence: jf236@cornell.edu

Insect susceptibility to a pathogen can be influenced by maturation of the insect and its reproductive history since tradeoffs can occur between immunity and physiological factors. Additionally, as insects age their ability to combat a pathogen can decrease through a process called immunosenescence. The fungal pathogen *Metarhizium brunneum* is being developed to control the Asian longhorned beetle (*Anoplophora glabripennis*), an invasive wood-borer. Since *A. glabripennis* take 1-2 weeks to mature after eclosion and can be long-lived, we wanted to determine how age, maturation and mating would impact their susceptibility to *M. brunneum*. Mature and older (both virgin and mated) and young (only virgin) beetles were inoculated with *M. brunneum*. The presence of *M. brunneum* in the hemolymph was quantified and beetles were monitored daily for death. There was a cost of reproduction for mature beetles but not the oldest beetles. We found no evidence of immunosenescence in older beetles and young males were more susceptible than mature or older beetles. Our results contrast with other studies which found immunosenescence in older invertebrates in many different systems. Our findings have important implications for understanding how mating and age can impact susceptibility to a fungal pathogen.

CONTRIBUTED PAPERS Thursday 8:00 – 10:00

Microbial Control 3

CONTRIBUTED PAPER Thursday 8:00 **137-STU**

Toxicity of *Bacillus thuringiensis* culture filtrate to *Meloidogyne incognita*

Jiaheling Qi^{1,2}, Daigo Aiuchi², Shin-ichiro Asano³, Masanori Koike²

¹The United Graduate School of Agricultural Sciences, Iwate University, Iwate Japan; ²Department of Agro-environmental Science, Obihiro University of Agriculture & Veterinary Medicine, Obihiro, Japan; ³Department of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Sapporo, Japan

Address for Correspondence: koike@obihiro.ac.jp

Bacillus thuringiensis has been used as an effective bio-insecticide because it produces the proteins Cry and Cyt, which are highly toxic to insects in certain situations. Root-knot nematode

Meloidogyne incognita, is an important plant parasite, it prefers to attack the root of its host plant and can cause extensive damage to a wide variety of economically important crops. Recently, *B. thuringiensis* strains have the nematicidal activity against root knot nematode was proved. So we focus on using *B. thuringiensis* as a nematicide to control the *Meloidogyne incognita*.

In this study, the nematicide effect of *B. thuringiensis* was identified by using bacteria culture filtrate (CF) treated second-stage nematode (J2) and egg sac. J2 suspensions (0.1 ml) were placed into wells of a 24-well tissue culture plate (50J2/well) containing 0.9 ml of bacteria CF. Liquid LB was used as the control. The effect of bacteria CF on the viability of J2 was determined after 12h. Bacteria CF from *B. thuringiensis* strains significantly increased the percentages of J2 motility compare to untreated control. Also, we inoculated bacteria CF of six strains of *B. thuringiensis* on *M. incognita* egg sac by co-culture method for 24h, and removed the egg sac into sterilized water. 3 days after inoculation, counted the quantity of hatched J2 in the bacteria CF and un-hatched eggs in the egg sac to calculate the hatchability of egg. *B. thuringiensis* strains reduced the hatchability of egg (from 28.4% to 63.90%) compare to the untreated control.

CONTRIBUTED PAPER Thursday 8:15 **138**

***Yersinia entomophaga*: A biopesticidal bacterium active against a wide range of insect pests**

Mark Hurst¹, Colin Ferguson², Sarah Mansfield¹, Richard Townsend¹, Sean Marshall, Sandra Jones¹, Jayanthi Swaminathan¹, David Wright¹, Michael Wilson³, Derick Wilson³, Maureen O'Callaghan¹

¹Innovative Farm systems, AgResearch, Lincoln Research Centre, New Zealand; ²Innovative Farm systems, AgResearch Invermay Agricultural Centre, New Zealand; ³Innovative Farm systems, AgResearch, Ruakura New Zealand

Correspondence: mark.hurst@agresearch.co.nz

The bacterium *Yersinia entomophaga* was isolated from larvae of the New Zealand grass grub, *Costelytra zealandica* (Coleoptera: Scarabaeidae), from New Zealand soils. *Y. entomophaga* has activity towards a number of Coleopteran and Lepidopteran pests with the main activity against members of the Scarabaeidae, where LD₅₀s of approximately 5 x 10⁴ bacteria/larva have been determined. Once ingested, death typically occurs within 2-5 day post infection. The main virulence determinant of *Y. entomophaga* is an insect active toxin complex termed the Yen-TC, that is highly active against members of the Scarabaeidae and the diamondback moth (*Plutella xylostella*). The bacterium can be economically fermented in large volumes, producing >1 x 10¹⁰ cells /mL within 24 hours. Media and tools have been defined to alter the levels of Yen-TC production and enabling the selective detection of the bacterium post application to the environment. Formulated *Y. entomophaga* has shown field efficacy when applied as a bait formulation against ground dwelling lepidoptera (*Wiseana* sp.). The same bait proved more effective (80% control) than the insecticide alpha-cypermethrin for the control of Black beetle (*Heteronychus arator*), a pasture pest originating from South Africa. Spray developments are in progress for another New Zealand pastoral pest the "plantain moth complex" family Geometridae, *Scopula rubraria* and *Epyaxa rosearia* which causes significant damage plantain crops. A range of *Y. entomophaga* based formulations and delivery systems (e.g. baits, sprays, granules, seed treatments) are being developed.

CONTRIBUTED PAPER Thursday 8:30 **139****Toxicological and protein characterization of *Bacillus sphaericus* C3-41 strain from Karnataka**Basavaraj S. Kalmath¹, A. Prabhuraj², Gajanan Katkar³, R. S. Giraddi¹, B. V. Patil¹^{1,4} College of Agriculture Bheemarayanagudi^{2,5} College of Agriculture Raichur³ Department of Biochemistry, Mysore UniversityCorrespondence: bskalmath@gmail.com

A native virulent *Bacillus sphaericus*C3-41(CP 000 817) was isolated and identified from Rabbanahalli village of Yadgiri district, Karnataka, India. The isolates from different geographical origins have different degree of toxicity and persistence in nature. There is vast scope to identify and further characterize native *B. sphaericus* against mosquito. we made an attempt to characterize the pathogenicity and protein analysis of native *B. sphaericus* against *C. quinquefasciatus*. *B. sphaericus* was subjected for pathogenicity against *C. quinquefasciatus* over various time of incubation, temperature and liquid media and protein analysis by SDS PAGE. Bioassay of *B. sphaericus* incubated for four day recorded the highest mortality of 100 per cent each at 750 ppm and 1000 ppm 48 hr after the treatments. Bioassay of *B. sphaericus* incubated at different temperature recorded, highest mortality at 30°C with 100 per cent 48 hr after the treatment. Among the media tested for bioassay, Egg yolk media recorded maximum mortality with 76.00 per cent and 100 per cent at 24 hr and 48 hr after treatments respectively. Further, the presence of large amount of *B. sphaericus* toxins proteins Bin A (41 KDa) and Bin B (52 KDa) were observed in SDS-PAGE, thus supporting its lethal potency against mosquito larvae. Therefore, it is highly likely that *B. sphaericus* isolate could serve as most potent bio-mosquitocide and play major role in dropping mosquito-vectored diseases.

CONTRIBUTED PAPER Thursday 8:45 **140****A new invasive biotype of the coconut rhinoceros beetle (*Oryctes rhinoceros*) has escaped from biological control by *Oryctes rhinoceros* nudivirius**Sean D. G. Marshall¹, Maclean Vaqalo², Aubrey Moore³, Roland J. Quituqua³, Trevor A. Jackson¹¹Innovative Farming Systems, AgResearch, Lincoln Research Centre, Christchurch, New Zealand; ²Land Resources Division, Secretariat of the Pacific Community, Suva, Fiji Islands; ³College of Natural and Applied Sciences, University of Guam, GU, USACorrespondence: sean.marshall@agresearch.co.nz

The coconut rhinoceros beetle (*Oryctes rhinoceros*; CRB) is a major pest of coconut and oil palm, but the discovery of *Oryctes rhinoceros* nudivirius (OrNV) in the 1960s has enabled the successful management of populations in Pacific Island Countries (PICs). 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 countries and 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 has been reported in the Pacific, being detected first in Tumon Bay in Guam 2007, followed by Port Moresby in PNG 2010, Honolulu in Hawaii 2013, and Honiara in Solomon Islands 2015. Additionally, Pacific areas with established CRB populations (e.g. Palau) have reported increased severity and frequency of CRB damage. Common to all these areas is the high incidence of severe palm damage, not seen since

the introduction of OrNV. Initial attempts to introduce OrNV into the Guam CRB population were unexpectedly unsuccessful, raising the possibility that the CRB-G population that invaded Guam could be tolerant or resistant to the commonly applied OrNV isolates. Analysis of several CRB populations has demonstrated that the CRB-G biotype is also found in Hawaii, Palau, and recently (February 2015) in Port Moresby (PNG), with Honiara (Solomon Islands) still to be confirmed. We will discuss current results in relation to what is known about these new invasions and potential implications for the future.

CONTRIBUTED PAPER Thursday 9:00 **141****Production of *Oryctes nudivirius* in DSIR-HA-1179 insect cell cultures in roller bottle systems**Gabriel Visnovsky¹, Charlotte Pushparajan¹, Juan D. Claus²¹Department of Chemical and Process Engineering, University of Canterbury, New Zealand; ²Lab. Virología, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, ArgentinaCorrespondence: gabriel.visnovsky@canterbury.ac.nz

The coconut rhinoceros beetle, an economically important pest of coconut palms, is effectively managed by application of its natural pathogen, the *Oryctes* nudivirius (OrNV). The traditional method of producing OrNV in infected larvae is labor intensive, and suffers from inconsistencies in virus titer and purity. *In vitro* production of OrNV in the susceptible and permissive anchorage-dependent DSIR-HA-1179 insect cell line can overcome these disadvantages. While production of OrNV in cell monolayers grown in T-flasks or Cell Factories is possible, the approach would not be cost-effective at industrial level. Roller Bottle Systems (RBS), with larger growth surface areas and capable of holding larger culture volumes in a more dynamic environment, would be a better option for scaling up purposes. DSIR-HA-1179 cells grown in 490 cm² RBS filled with 60 ml of TC-100 culture medium added with 10% fetal bovine serum yielded a maximum of 1.5×10^6 cells/ml, which was comparable to yields reached in T-flasks under the same culture conditions (1.7×10^6 cells/ml). Infection in RBS of cells in their early exponential growth phase (5×10^5 cells/ml) at multiplicity of infection of 1 TCID₅₀/cell, produced OrNV volumetric and cell-specific yields of 2.1×10^8 TCID₅₀/ml and 250 TCID₅₀/cell respectively, which was an improvement over those produced under identical culture and infection conditions in T-flasks (volumetric and cell-specific yields of 6.8×10^7 TCID₅₀/ml and 102 TCID₅₀/cell, respectively). Thus, the roller bottle system has proven to be a robust alternative for the industrial production of OrNV.

CONTRIBUTED PAPER Thursday 9:15 **142****Control of *Chrysodeixis includens* (Walker) and *Anticarsia gemmatalis* Hübner with *Bacillus thuringiensis*, chlorantraniliprole, and a mixture of PsiSNPV and AgMNPV**Daniel R. Sosa-Gómez

Embrapa Soybean, Londrina, PR, Brazil

Correspondence: daniel.sosa.gomez@embrapa.br

In Brazil, infestations of soybean looper (SBL) (*Chrysodeixis includens*) and velvetbean caterpillar (VBC) (*Anticarsia gemmatalis*) generally occur simultaneously in soybean crops. Due to the high tolerance of SBL to insecticides, it is usually harder to control it than VBC. To verify the efficacy of the simultaneous application of viruses that infect both insect species, a field trial was conducted in January 2015 using the following treatments: 1) control, tap water; 2) chlorantraniliprole = 10 g a.i.ha⁻¹; 3) *Bacillus thuringiensis* = 750 g.ha⁻¹; 4) AgMNPV = 1.5×10^{11} (occlusion bodies

- OB.ha⁻¹) + PsiSNPV = 6×10¹² OB.ha⁻¹. Applications were performed in 200 L water.ha⁻¹ after 5:00 PM. Chlorantraniliprole was very efficient to control VBC, but not SBL, while *B. thuringiensis* reduced both populations. Both viruses significantly reduced SBL and VBC populations seven and 10 days after spraying, respectively. After 15 days, the number of larvae in the plots treated with the viruses was lower (VBC < 6.9 and SBL < 0.1) and statistically similar to the control plots. Defoliation was significantly reduced in all treated plots compared to the control plots during the 18 days of the field trial. Defoliation ranged from 10% (one week after application) to 18% (18 days after application) in the NPV treated plots, whereas in the control plots it ranged from 14% to 31% after one week and 18 days after application, respectively. Accumulated defoliation in the plots treated with *B. thuringiensis* and chlorantraniliprole was less than 10%.

CONTRIBUTED PAPER Thursday 9:30 **143**

Fungal endophytes as first line of defense against the bean stem maggot *Ophiomyia phaseoli* (Tyron) on *Phaseolus vulgaris* (L.)
Beritah Mutune^{1,2}, Sunday Ekesi¹, Saliou Niassy¹, Vivienne Matiru²,
Christine Bir², Nguya K. Maniania¹

¹International Centre of Insect Physiology and Ecology (icipe), P. O. Box 30772-00100, Nairobi, Kenya; ²Jomo Kenyatta University of Agriculture and Technology (JKUAT), P.O. Box 62000-00200, Nairobi, Kenya

Correspondence: nmaniania@icipe.org

Common bean, *Phaseolus vulgaris*, is an important food and cash crop in Africa. However, its production is seriously affected by the bean stem maggot (BSM), *Ophiomyia* spp. Larval feeding and tunneling interfere with nutrient transport and creates avenues for entry of disease organisms. The presence of BSM pupae in the plant results in the swelling and rotting of the seedlings, causing up to 100% loss. The management of BSM is difficult because of their cryptic behavior. We evaluated the ability of 11 fungal isolates to colonize bean plants and the effects of inoculation on BSM feeding and oviposition, pupation and adult emergence. All fungal isolates were able to colonize different bean plant parts (root, stem and leaves), except isolates of *Metarhizium anisopliae* and one isolate of *Beauveria bassiana*. Colonization varied significantly between the fungal isolates ($F_{11,165} = 18.7$, $P < 0.0001$) and plants parts ($F_{2,312} = 8.5$, $P = 0.0002$). *BSM feeding and oviposition were significantly reduced* ($F_{11,165} = 19.82$, $P < 0.0001$) *in all the fungus-inoculated bean plants which in turn affected pupation* ($F_{11,165} = 17.28$, $P < 0.0001$) *and adult emergence* ($F_{11,165} = 10.46$, $P < 0.0001$) as compared to the control. *Metarhizium anisopliae* 20 outperformed the other isolates in interfering with BSM lifecycle. Although *M. anisopliae* 78 recorded a high number of punctures similar to the control, a significant reduction in the number of pupae and adult emergence was observed, suggesting possible BSM growth inhibition. This study demonstrates clearly that fungal endophytes can be considered as a first line defense strategy against BSM in East Africa.

CONTRIBUTED PAPER Thursday 9:45 **144**

Performance of three Indian isolates of *Beauveria bassiana* (Balsamo) Vuillemin and three commercial mycoinsecticides against three developmental stages of *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae)

Mohammed I. Elbashir¹, P. Bishwajeet², K. Shankarganesh³, P. Sharma⁴

¹Department of Bio pesticides and Bio fertilizers- Environment and Natural Resources and Desertification Research Institute, P.O. Box 6096, Khartoum, Sudan; ^{2,3}Biological Control Laboratory, Division

of Entomology, Indian Agricultural Research Institute, New Delhi-110012; ⁴Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi-110012

Correspondence: fatalooue@yahoo.com

Oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) has been identified as one of the three most important agricultural pests in South East Asia. In India, it causes losses up to 80% on mango alone. In this study three isolates of *B. bassiana* and three commercial mycoinsecticides were screened against larva, pupa and adults of *B. dorsalis*. The isolates ITCC No. 6628 and B. NCIPM were found pathogenic to adult stage with LC₅₀ 2.5×10⁵ and 7.5×10⁵ conidia /ml, respectively. While, ITCC No. 6645 found effective against adult stage at 1.2×10⁹ and 3rd larval stage with LC₅₀ 9×10⁷ conidia /ml. However, the three commercial mycoinsecticides prove their efficacy only on adult stages with 26.6%, 40% and 46.6% mortality for, Bio-power[®], Bio-magic[®], Bio-catch[®], respectively. Significant differences between treatments and control were recorded accompanied by observation of mycosis on the cadavers.

SYMPOSIUM Thursday 14:00 – 16:00

Synergies Enabling the Registration and Adoption of Biological Pest Controls - The Role of Governments, and Academic Programmes and Industry

SYMPOSIUM Thursday 14:00 **145**

Facilitating the registration and adoption of biological pest controls in Canada

Tobias Laengle

Pesticide Risk Reduction Program, Agriculture and Agri-Food Canada, St. John's, NL, Canada

Correspondence: tobias.laengle@agr.gc.ca

Biological pest control products, which include microbials, pheromones, and other non-conventional pest controls, can help growers develop and maintain advanced Integrated Pest Management practices that complement ecosystem services provided by intact agricultural ecosystems. Recognizing the limited availability of these types of products in Canada, in 2005 Agri-Food Canada's Pesticide Risk Reduction Program (PRRP) began taking an active role in encouraging and assisting companies with the registration of biological pest controls. Meanwhile, biological pest control products, also referred to as biopesticides, have been gaining momentum worldwide, and many of the major players in the agriculture inputs industry have entered the biopesticides field with significant investments. As part of the symposium "Synergies enabling the registration and adoption of biological pest controls – the role of governments, academic programmes, and industry", this talk will outline how the PRRP contributes to and builds on this global trend. The PRRP optimizes growers' ability to access and use innovative new technologies by providing support along the innovation chain by (i) supporting research on biopesticides development, (ii) assisting and advising AAFC researchers with the commercialization of products, (iii) providing regulatory support to priority biopesticides identified through a grower-directed process, and (iv) supporting IPM projects to demonstrate the successful integration of biopesticides into integrated production systems. Successful examples will be used to illustrate this work of Pesticide Risk Reduction Program.

SYMPOSIUM Thursday 14:25 **146****The IR-4 biopesticide and organic support program**Michael Braverman, *William Barney*

Interregional Research Project Number 4 (IR-4), Rutgers University, Princeton, NJ, USA

Correspondence: barney@aesop.rutgers.edu

The IR-4 Project is funded by the USDA agencies NIFA and ARS and receives support from the directors of state agricultural experiment stations. IR-4 is an applied research program whose mission is to assist specialty crop producers obtain safe and effective pest control products. The program was initiated in 1963 and historically has focused on pest management tools for use on specialty crops or for minor uses on major crops. IR-4 broadened its scope in 1982 to include research leading to registration of a wide range of biopesticides including microbials, biochemicals, and genetically altered or transgenic plants. The program is committed to developing alternative pest control products on specialty food crops and ornamentals by working cooperatively with public and private sector individuals and organizations. IR-4 interacts with the USDA, EPA, and AAFC to determine the requirements for registration of proposed uses. The program has the resources to develop research protocols, assist with Experimental Use Permits, coordinate and fund field and greenhouse research, assist in the development of Tier I toxicology and non-target organism waivers, arrange pre-registration meetings and prepare data packages for submission to the EPA. The IR-4 Project has transitioned from an annual biopesticide grant program to a directed research program to enable biopesticide research on critical needs in fruit, vegetables, ornamentals, public health and other research areas

SYMPOSIUM Thursday 14:50 **147****How does academia contribute to registration and adoption of biological control agents, a European perspective**

Jørgen Eilenberg

Department of Plant and Environmental Sciences, University of Copenhagen, Frederiksberg C, Denmark

Correspondence: jei@plen.ku.dk

In the EU, microbiological control agents are currently registered by Regulation (EC) No 1107/2009, while macrobiological control agents are not included in this regulation. The active substance (based on an isolate of a microorganism), is approved by the EU and the decision applies to all 28 member states. The formulated product is, however, authorised at national level. The complete procedure is often long lasting. EU has an explicit wish to support IPM, including the use of biological control. Academia in EU can contribute to registration and adoption of biological control in several ways:

- 1) Joint research and innovation programs involving several research teams from different countries as well as private companies. Collaboration between research teams and SMEs (small to medium sized enterprises) is strongly encouraged in projects financed by the EU.
- 2) Networks and working groups related to the subject and sometimes even initiated with the explicit purpose to contribute to registration and adoption of biological control.

I will give a few examples of both types of activities. An example of particular relevance is the working group established in 2013 in order to suggest to the EU how to implement the 'low risk' concept for compounds to be approved. This work includes that we suggest to the EU how to define criteria for microorganisms to qualify for being considered as 'low risk' and also, how a 'fast

lane' for registration can be implemented for such microorganisms.

SYMPOSIUM Thursday 15:15 **148****Perils and pitfalls of product development and commercialization: An industry perspective**

Randy Martin

Manager, R&D Pipeline, Valent BioSciences, Inc.

Correspondence: Randy.Martin@valentbiosciences.com

The process of developing a crop protection product for use in agriculture is replete with hazards. Naturally, companies want to minimize their risk, while increasing their chances at success. In order to deploy their resources in the most efficient manner, companies must precisely evaluate candidate technologies, and accurately define target markets and realistic sales goals at the earliest stages of the development process. Most companies deploy a process with well-defined phases and gate reviews to help principals make decisions along the way. During the development process there are potential avenues for synergy with government regulatory agencies as well as academic research institutions; these will be discussed.

CONTRIBUTED PAPERS Thursday 14:00 – 15:30

Viruses 5CONTRIBUTED PAPER Thursday 14:00 **149****Peritrophic matrix proteomics of the cotton leaf worm, *Spodoptera littoralis* (Lepidoptera: Noctuidae): Targets for pathogens infecting *per os***Umut Toprak¹, Dwayne Hegedus², Esengul Ozdemir¹, Doug Baldwin², Cathy Coutu², Diney Bekkaoui², Serife Bayram¹, M. Oktay Gürkan¹¹Ankara University, Faculty of Agriculture, Dept. of Plant Protection, Ankara, Turkey; ²Agriculture and Agri-Food Canada, Saskatoon Research Centre, Saskatoon, SK CanadaCorrespondence: utoprak@agri.ankara.edu.tr

Peritrophic matrix (PM) is a porous structure lining the midgut in many insects and is composed of chitin and proteins. PM serves as a barrier that protects the midgut epithelial cells from pathogen entrance, as well as other roles. Pathogens must pass through the PM to initiate infection *per os* and several integral PM proteins may be targeted by these pathogens. *Spodoptera littoralis* (Lepidoptera: Noctuidae), commonly known as the Egyptian cotton leaf worm, is a destructive major pest of a variety of economically important vegetables and field crops in Mediterranean countries. In the current study, PMs were dissected from 5th instar *S. littoralis* larvae and proteins associated with the PM were extracted using a solution containing 2.5% SDS, 5% β-mercaptoethanol and 500 mM NaCl. The extracted proteins were exposed to liquid chromatography-tandem mass spectrometry (LC-MS/MS) and peptide masses were determined in a *S. littoralis* midgut genomic library. This analyses revealed the presence of various peritrophins, enzymes involved in chitin metabolism, digestive enzymes, as well as other enzymes and proteins. Expression of two groups of genes encoding mucin peritrophins and chitin deacetylases was examined by droplet digital PCR in the presence of baculovirus infection. These analyses revealed a differential expression pattern of these genes, suggesting they are involved in defense against baculovirus infection.

CONTRIBUTED PAPER Thursday 14:15 **150****Analysis of occlusion derived virus infection in tissue culture using gene knock out viruses of the *per os* infectivity factors and GP64**David A. Theilmann¹, Leslie G. Willis¹, Michael Weis¹, Cam Donly², Dwayne D. Hegedus³, Martin A. Erlandson³¹Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Summerland, British Columbia, Canada V0H 1Z0;²Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, London, Ont., Canada; ³Saskatoon Research Center, Agriculture and Agri-Food Canada, Saskatoon, SK, CanadaCorrespondence: david.theilmann@agr.gc.ca

Infection of lepidopteran cells by the baculovirus AcMNPV results in two virion forms, budded virus (BV) and occlusion derived virus (ODV). BV is required for the systemic spread of the virus and attaches to and enters cells by receptor mediated endocytosis mediated by the viral envelope glycoprotein GP64. ODV are required inter-host transmission and bind and enter the host columnar midgut epithelial cells via a receptor mediated membrane fusion event mediated by at least seven *per os* infectivity factors (PIFs). Previous studies have shown that both BV and ODV are infectious for tissue culture cells. Compared to BV however, ODV have very low infectivity for tissue cells and it is unknown if it requires the PIF complex. To determine if tissue culture infection by ODV requires the PIF complex we generated seven PIF gene knockout viruses, *pif0-6*. In addition, to ensure any infection observed was not due to GP64 mediated processes each of the *pif* knockout viruses was generated in a genomic backbone that contained a deletion of *gp64*. Occlusion bodies were purified transfected cells and ODV were alkali liberated and used for infection assays. Tissue culture infection of ODV, derived from *pif-gp64* double knockouts, was compared to ODV isolated from *gp64* knockout viruses. Initial results with *pif0* and *pif4* knockouts have shown that infectivity was very low for both *pif* knockouts and the control virus, but no difference was observed in infection levels. These initial results suggest that the ODV infection of tissue culture cells do not utilize the PIF complex and an alternate entry mechanism must exist.

CONTRIBUTED PAPER Thursday 14:30 **151****Inhibition of *Cotesia vanessae* development in *Trichoplusia ni* larvae infected with alphabaculoviruses from *Mamestra configurata***Martin A. Erlandson^{1,2}, Stephanie Harris¹, Edyta Sieminska², Doug Baldwin¹, Dwayne D. Hegedus¹, David A. Theilmann³¹Agriculture and Agri-Food Canada, Saskatoon Research Center, Saskatoon, SK, Canada; ²Department of Biology, University of Saskatchewan, Saskatoon, SK, Canada; ³Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, Summerland, BC, CanadaCorrespondence: martin.erlandson@agr.gc.ca

Typically the interaction between parasitoids and baculoviruses exploiting the same host is a competitive one and the survival of the parasitoid larvae is dependent on the timing of parasitization and/or infection. The negative impact on parasitoid development is typically a result of baculovirus infection exhausting the metabolic resources of the host before the parasitoid completes its larval development. However, there are exceptions to this general case and examples of betabaculovirus and entomopoxvirus infections causing direct toxicity to or inhibition of parasitoid development have been described. It has been demonstrated that a protein from the hemolymph of virus-

infected hosts is responsible for inhibition of development and killing of the parasitoid larvae. Among a large collection of baculoviruses isolated from geographic populations of bertha armyworm (*Mamestra configurata*) are strains of both MacoNPV-A and MacoNPV-B species that contain cassettes of four to six genes that are highly homologous to *Xestia c. nigrum* granulovirus (XecnGV). MacoNPV-A and MacoNPV-B isolate infections in *T. ni* larvae were assessed for negative impacts on the development of the braconid parasitoid *Cotesia vanessae*. Those MacoNPV viruses containing a cassette of XecnGV genes inhibit the development of *C. vanessae* larvae in *T. ni* larvae. Development of AcMNPV recombinant viruses showed that a single XecnGV gene homologue in MacoNPV viruses is responsible for the inhibition of parasitoid development. In vitro assays showed that the purified recombinant protein is toxic to *C. vanessae* larvae. Potential gene flow between distantly related viral species as well as competition between baculoviruses and parasitoids play a significant role in bertha armyworm ecology.

CONTRIBUTED PAPER Thursday 14:45 **152****First successful elimination of an insect virus, *Glossina pallidipes* salivary gland hypertrophy virus, from an insect factory: A model for managing insect viruses in insect factories for food and feed**

Adly Abd-Alla

Insect Pest Control Laboratory, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, Vienna, Austria

Correspondence: a.m.abd-alla@iaea.org

Tsetse flies (Diptera; Glossinidae) are naturally infected by *Glossina* hytrosavirus (family Hytrosaviridae), a large dsDNA virus pathogenic specifically to the *Glossina* spp. The virus infections are largely asymptomatic but in *G. pallidipes*, asymptomatic infection can convert to symptomatic infection that is characterized by salivary gland hypertrophy syndrome (SGH), leading to reproductive dysfunction of infected flies which lead in several cases to the collapse of insect factory. Over the last decade researches were conducted to develop a virus management strategy to control and/or eliminate this virus from tsetse mass rearing facilities. The management strategies relies on (i) collecting information on the virus transmission, (ii) understanding tsetse biology and rearing system (iii) using available antiviral drugs to reduce the virus replication and infection, (iv) modifying tsetse feeding system to a clean feeding system to interrupt the virus transmission and (v) combining the use of clean feeding system with antiviral drugs. This management system leads to the elimination of the salivary gland symptom from the infected colony after two years post implementation. Continuing the clean feeding system resulted in the elimination of the virus infection as demonstrated with the absence of the virus infection when tested with PCR at 3 year post implementation. This management system could be used as a model for developing virus management strategy for insect factory for food and feed.

CONTRIBUTED PAPER Thursday 15:00 **153****Impact of valacyclovir on the pathology of hytrosavirus in *Musca domestica***D. G. Boucias¹, C. Geden², A. M. M. Abd-Alla³¹Entomology and Nematology Department, University of Florida, Gainesville, USA; ²Center for Medical, Agricultural and Veterinary Entomology, USDA, Gainesville, USA; ³Insect Pest Control Laboratory, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, AustriaCorrespondence: paths@ufl.edu

Prior reports have demonstrated that orally delivered valcyclovir suppresses hytrosavirus (GpSGHV) levels in *Glossina pallipides* colonies. Due to the complex nature of this insect-virus interaction, we decided to conduct parallel studies using the MdSGHV/*Musca domestica* system. Unlike GpSGHV, the MdSGHV expresses an acute infection within three days post-challenge, showing both typical SGH symptoms and virus-induced female sterility in 100% of the treated flies. Accordingly, a series of bioassays were conducted. Flies were injected with valcyclovir and MdSGHV then fed adult food and water amended with valcyclovir. An array of controls determined the impacts of the virus or valcyclovir on female fitness. A second set of bioassays were conducted that included 48h pretreatment of females with a 10% sucrose with and without valcyclovir. These flies were subsequently injected with the virus + valcyclovir cocktail and fed valcyclovir-amended water and adult food. To date, observations have demonstrated that the valcyclovir, at best, only partially suppresses the onset of SGH symptoms. Females treated with valcyclovir and virus fall into three categories: normal SGH, partial or aborted SGH, and no visible SGH. It should be noted that those that failed to display hypertrophy failed to develop eggs; these results suggest that the immediate early genes of MdSGHV may be mediating the virus-induced female sterility. It should be noted that the valcyclovir controls produced a normal complement of eggs, and all virus controls produced pronounced SGH and total female sterility. Ongoing experiments using a combination of conventional qPCR and RT-qPCR will provide quantitative data to these bioassays.

CONTRIBUTED PAPER Thursday 15:15 **154**

Pathogen-host interaction of deformed wing virus (DWV) and the honey bee (*Apis mellifera*)

Sebastian Gisder, Elke Genersch

Institute for Bee Research, Molecular Microbiology and Bee Pathology, Hohen Neuendorf, Germany

Correspondence: gisderse@rz.hu-berlin.de

Deformed wing virus (DWV) is a viral pathogen of the European honeybee *Apis mellifera* and is present in the majority of honey bee colonies worldwide. Vertical transmission by larval food, eggs or drone sperm causes true covert infections and progeny emerge without symptoms of a DWV infection. Outbreaks of clinical DWV-infections are associated with Varroa mite (*Varroa destructor*) infestation and cause overt infections with characteristically symptoms such as crippled wings and shortened abdomens. Varroa mites feed on honey bee pupae's hemolymph and ingest DWV. Since it is a highly variable virus, different variants develop or a certain virus variant can propagate to a high copy number in mite cells. The DWV mutants were transmitted to the brood when the mite feeds again on honey bee larvae. Emerging bees have elevated virus levels and overt infections always correlate with the detection of DWV-RNA in brains of crippled bees. But also asymptomatic adults can be DWV-positive in their brains leading to the assumption that several DWV-mutants may lead to chronic overt infections with sublethal impairments such as learning disabilities of adult honey bees.

CONTRIBUTED PAPERS Thursday 14:00 – 16:15

Bacteria 3

CONTRIBUTED PAPER Thursday 14:00 **156**

Cyt1Aa-BinA chimera highly toxic to anopheline, aedine, and culicine larvae including those tolerant or resistant to

Lysinibacillus sphaericus

Dennis K. Bideshi^{1,2}, Hyun-Woo Park^{1,2}, Robert H. Hice¹, Margaret C. Wirth¹, Brian A. Federici^{1,3}

¹Department of Entomology, University of California, Riverside, USA; ²Department of Natural and Mathematical Sciences, California Baptist University, Riverside, USA; ³Institute for Integrative Genome Biology, University of California, Riverside, USA

Correspondence: brian.federici@ucr.edu

Two naturally occurring mosquitocidal bacilli, *Bacillus thuringiensis* subsp. *israelensis* (Bti) and *Lysinibacillus sphaericus* (Ls), are the active ingredients of commercial larvicides used for controlling nuisance and vector mosquitoes. Bti's high insecticidal activity is due to synergistic interactions among its four major proteins (Cry4Aa, Cry4Ba, Cry11Aa, and Cyt1Aa), whereas Ls's principal toxicity is caused by the binary mosquitocidal crystal, Bin, containing BinA, a toxin domain, and BinB a midgut-binding domain. Although used in many countries for over three decades, resistance to Bti is very rare. However, Bin resistance levels greater than 20,000-fold have occurred where Ls has been used intensively for mosquito control. Bti's Cyt1Aa is a lipophilic protein of low toxicity that binds to midgut microvilli, and in previous studies we showed it delays the evolution of resistance to Bti Cry proteins and Ls Bin, and can overcome resistance to these when selected for in the laboratory. In the present study, we assayed Cyt1Aa as a broad-spectrum targeting domain by fusing it to BinA. Here we show that the Cyt1Aa-BinA chimera accumulates in a parasporal body in 4Q7, an acrySTALLIFEROUS strain of Bti, and has remarkably high toxicity to larvae of major mosquito vectors, namely, *Anopheles gambiae*, *An. stephensi*, Bin-sensitive and Bin-resistant strains of *Culex quinquefasciatus*, and *Aedes aegypti*, the latter which is not normally sensitive to Ls Bin. Our results suggest Cyt1Aa may prove useful as a targeting protein for other insecticidal proteins used to control dipteran vectors and possibly for dipteran and non-dipteran agricultural pests.

CONTRIBUTED PAPER Thursday 14:15 **157**

Effect of single versus multiple promoters and a high plasmid copy number on the synthesis and assembly of Cyt1Aa crystals in *Bacillus thuringiensis*

Hyun-Woo Park¹, Robert H. Hice², Brian A. Federici²

¹Department of Entomology, University of California, Riverside, and Department of Natural and Mathematical Sciences, California Baptist University, Riverside, USA; ²Interdepartmental Graduate Programs in Microbiology and Cell, Molecular, and Developmental Biology, University of California, Riverside, USA

Correspondence: hyunwoo.park@ucr.edu

Cyt1Aa is a major mosquitocidal protein synthesized during sporulation of *Bacillus thuringiensis* subsp. *israelensis*, making up more than 50% of this species parasporal body. This high level of Cyt1A synthesis is due to several molecular traits including three strong sporulation-dependent promoters, a strong transcription termination sequence, and an associated 20-kDa helper protein. Although Cyt1Aa's toxicity is low compared to this species Cry proteins, it nevertheless plays an important role in the biology of *B. thuringiensis* subsp. *israelensis* in that it synergizes the toxicity of the latter proteins, namely Cry11Aa, Cry4Aa and Cry4Ba, and delays the evolution of resistance to these. In the present study, we evaluated the effects of using different *cyt1Aa* promoter combinations and plasmid copy number on synthesis of Cyt1Aa. Using the 4Q7 (plasmid-cured) strain of *B. thuringiensis* subsp. *israelensis* as an experimental host, a plasmid copy number of two or three yielded no Cyt1Aa, whereas a copy number of four

yielded only small crystals, even when expression was driven by one of the wild type promoters. However, using all three wild type promoters and a plasmid copy number of 20 yielded Cyt1A crystals 10-fold larger than those produced by one promoter and a plasmid copy number of four. In general, high levels of Cyt1Aa synthesis resulted in a significant reduction of spores per unit medium and imperfectly formed crystals. Cyt1Aa crystal synthesis using the same expression systems was also evaluated using a *B. thuringiensis* subsp. *israelensis* mutant strain that does not produce Cyt1Aa. Very similar results were obtained when the same expression constructs were evaluated in this mutant Cyt1A-minus strain.

CONTRIBUTED PAPER Thursday 14:30 **158**

The island of mosquitocidal toxins in *Bt* strain 2160-1 identified by whole genome sequencing and proteogenomic analysis

Jim X. J. Fang^{1,2,3}, *Y. Zhou*², *P. T. Gong*², *Y. J. Wei*³, *Y. Zhang*³

¹The HITAR Institute Canada Inc., Richmond, Canada; ²Hainan

Institute of Tropical Agricultural Resources, Sanya, China;

³Institute of Life Sciences, Jiyang College of Zhejiang A&F

University, Zhuji, China

Correspondence: jim.x.j.fang@hitar.org

The *Bacillus thuringiensis* strain S2160-1 has previously been identified as being highly toxic to mosquito larvae and a viable alternative to strains currently used commercially to control these insects. In a genome-wide scan, we predicted a sets of toxins in plasmids clustering in the region where likely formed a toxic island that included 10 toxin-like genes. Proteogenomic analysis proved the presence of the island of mosquitocidal toxins in *B.t.* Strain 2160-1, which included newly discovered toxins and previous identified five putative insecticidal toxin genes (Cry4Cb, cry30Ea, cry30Ga, cry50Ba and cry54Ba) in this strain. Our findings might provide new insights into the toxic protein island of *Bacillus thuringiensis* and the role of pathogenicity.

CONTRIBUTED PAPER Thursday 14:45 **159**

Engineering of *Bacillus thuringiensis* Cry proteins to improve the activity against western corn rootworm

Ruth Cong, *Hana Ali*, *Michi Izumi Wilcoxon*, *Yi Zheng*, *Jingdong Hou*, *Mark Nelson*¹, *Ericka Bermudez*, *Mark McDonald*, *Takashi Yamamoto*

Plant Protection, Ag Biotechnology, DuPont Pioneer, Hayward, CA and Wilmington, DE

Correspondence: ruth.cong@Pioneer.com

A new 133-kDa *Bacillus thuringiensis* Cry protein active against Western corn rootworm (WCRW) was discovered. This protein was designated as Cry8Hb1 by *Bacillus thuringiensis* Toxin Nomenclature Committee. The peptide sequence deduced from the cloned gene indicates a typical three domain-type Cry toxin. Trypsin digestion of Cry8Hb produced the 65-kDa matured toxin along with a 55-kDa fragment. This trypsin digestion pattern is similar to those of Coleoptera-active Cry proteins such as Cry3. The 55-kDa fragment is a result of nicking the Alpha 3-4 loop of Domain I by trypsin. Unexpectedly, it was found that the activity of Cry8Hb against WCRW was enhanced significantly by fusing *Escherichia coli* maltose binding protein (MBP) to the 75-kDa N-terminal portion of Cry8Hb including the leader sequence and matured toxin. Similar activity improvements were observed with the other corn rootworm-active proteins such as Cry8Bb. It appears the activity enhancement is a result of increased solubility of the MBP fusions of those Cry toxins at pH 7 or slightly acidic pH similar to the environment within the corn rootworm midgut. Solubility of a *Bt* Cry toxin can be modified by DNA

shuffling technology introduced by Stemmer (1994, *Nature*, 370:389-391). To evaluate this concept, a synthetic Cry3-type protein termed IP3-1 was designed by computer-aided protein-engineering using Cry3-family protein sequences and used as the starting material to conduct single-gene, mutagenesis DNA shuffling. The shuffling produced six highly active IP3 variants showing good solubility in a neutral pH buffer.

CONTRIBUTED PAPER Thursday 15:00 **160**

Laboratory-selected western corn rootworm colony resistant to mCry3A

Jian-Zhou Zhao, *Meghan Oneal*, *Nina Richtman*, *Analiza Alves*, *Stephen Thompson*

DuPont Pioneer, Johnston, U.S.A

Correspondence: Joe.Zhao@Pioneer.com

Bt maize products expressing various insecticidal Cry protein genes for western corn rootworm (*Diabrotica virgifera virgifera*, WCRW) management have provided significant value to growers over the past 10 years. Developing insect colonies resistant to *Bt* traits through laboratory selections can provide valuable resources for research and development of novel traits. We developed a laboratory-selected WCRW colony with a high level of resistance to mCry3A using purified protein and maize events expressing high levels of mCry3A. The resistance ratio (RR) to mCry3A was >90-fold in diet based bioassays. The beetles of the resistant colony were used to make reciprocal crosses with a susceptible laboratory colony to test the inheritance of resistance. Analysis of 12-day relative survival results for the F1 larvae indicated that resistance to mCry3A was inherited autosomally and was incompletely recessive.

CONTRIBUTED PAPER Thursday 15:15 **161**

A new Cry1Ac toxin of *Bacillus thuringiensis* highly toxic to *Manduca sexta* and *Trichoplusia ni*

*Yaritza Reinoso-Pozo*¹, *Cristina Del Rincón-Castro*², *Jorge E. Ibarra*¹

¹Department of Biotechnology and Biochemistry, CINVESTAV-

Irapuato, Mexico; ²Life Sciences Division, University of

Guanajuato, Irapuato, Mexico

jibarra@ira.cinvestav.mx

For the control of insects pest in agriculture different strategies to reduce the environmental impact produced by chemical insecticides have been used. *Bacillus thuringiensis* is a Gram-positive bacterium which produces crystal inclusions composed by Cry proteins that are toxic to a limited number of target insect larvae. In this study was performed a characterization of a native *B. thuringiensis* strain called LBIT-1200 which showed high toxicity in preliminary experiments against *Trichoplusia ni* and *Manduca sexta* larvae. Analysis of the flagellin sequence, plasmids pattern and REP-PCR showed that LBIT-1200 strain belongs to the *kurstaki* serovar and microscopy investigation showed the presence of bipyramidal crystals. The presence of *cry* genes in LBIT-1200 was studied using a PCR strategy with a set of degenerate primers and only one *cry1Ac* gene was identified. The LC₅₀ using spore-crystal mixture of LBIT-1200 against *M. sexta* y *T. ni* was 3.581 and 11.458 ng/cm², respectively. A comparison with the reference strains *B. thuringiensis kurstaki* HD-73, which also contains only a *cry1Ac* gene, showed much lower toxicity levels than LBIT-1200 (21.873 and 108.671 ng/cm², respectively). Sequence analysis of the Cry protein derived from LBIT-1200 *cry1Ac* gene showed three different amino acid substitutions, two in the toxic region (domain III) and one in the non-toxic C-terminal region of the protein. These substitutions are not present in the rest of the Cry1Ac proteins reported to date. Site direct mutagenesis experiments

were performed to determine if these substitutions are related to the high toxicity of the LBIT-1200 strain.

CONTRIBUTED PAPER Thursday 15:30 **162**

Rapid evolution and genetic basis of resistance to Cry1F in a lab-selected Asian corn borer and its cross-resistance to other Cry toxins

Yueqin Wang, Yidong Wang, Zhenying Wang, Kanglai He
State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protect, Chinese Academy of Agricultural Sciences, Beijing, China
Correspondence: hekanglai@caas.cn

The Asian corn borer, *Ostrinia furnacalis* (Guenée), is one of the most economic insect pests of maize targeted by Bt maize that has been tested in confined field trials in China. The evolution of resistance by the target insect and its management strategies should be clarified before Bt maize commercialization. It is important for characterizing resistant strains, which will provide the only way to empirically validate proposed management strategies. ACB-FR, a strain of the Asian corn borer, originated from field collection of Xi'an, Shaanxi Province was selected for resistance by exposure to Cry1F toxin incorporated into artificial diet in the laboratory. The susceptibility of selected strain to Cry1F toxin was declined with the selection pressure increased. The selected strain developed more than 52-fold resistance to Cry1F after 14 generations of selection. However, it showed lower level of cross-resistance to Cry1Ab, Cry1Ac and Cry1Ah, but it did not cross-resistance to Cry1e. Bioassay results of F₁ reciprocal crosses between ACB-FR and unselected strains showed that the inheritance of resistance to Cry1F was autosomal without maternal effect. Dominance D calculated on the basis of LC₅₀s suggested that resistance to Cry1F toxin was incompletely recessive. Backcrossing studies indicated that resistance to Cry1F toxin was polygenic in ACB-FR.

CONTRIBUTED PAPER Thursday 15:45 **163**

Evaluation of Bt corn with stacked genes for resistance to the Asian corn borer

Tiantao Zhang, Fan Jiang, Zhenying Wang, Kanglai He
State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China
Correspondence: klhe@ippcaas.cn

Transgenic *Bacillus thuringiensis* (Bt) crop could provide an effective way to control the insect pests. Preventing insect pests from developing resistance to Bt toxins produced by transgenic crops is a major challenge for agriculture. Theoretical models suggest that plants including two dissimilar Bt toxin genes have the potential to delay resistance more effectively than single-toxin plants used sequentially or in mosaics. To test these predictions, transgenic corn plants carrying Cry1Ac or Cry1e were obtained, and breeding stacked to two gene corn. To evaluate the resistance of the stacked Bt corn to Asian corn borer (ACB), laboratory bioassays were carried out by exposing susceptible or different resistant ACB neonates to fresh Bt corn whorl, immature tassels, fresh silks, ears and husk. The mortality of ACB neonates indicated that multiple transgenic hybrids have more effective insecticidal efficacy to both susceptible and resistant ACB. Furthermore, field trials were also conducted with artificial infestations of ACB at mid whorl and silk stages.

Bonning, Bryony C.	73	Choi, Jae Young	BA-13-STU, VI-18
Boucher, Matthew	106-STU, VI-14	Christensen, Skylar	FU-8
Boucias, D.G.	153	Chu, Xin-Ling	94
Boyle, Brian	119	Chubinishvili, Mariam	NE-4-STU
Braverman, Michael	146	Clark, K. Fraser	67
Bravo, AlejanDra	BA-6	Claus, Juan D.	141
Bravo, Lorena K.B.	MC-16	Clem, Rollie J.	30
Broadley, Hannah J.	106-STU	Coghlin, Paul	BA-4-STU
Brommmit, Angela	77	Cohen, Alejandro	119
Brookes, Jenny J.	FU-27-STU	Collier, Karin	FU-14, FU-16
Brown, Markus	FU-4	Cong, Ruth	159
Brownbridge, Michael	25, 29, 77	Corallo, Ana B.	FU-20
Buchter, Stefanie	FU-14	Cortés-Martinez, C.I	NE-1
Buitenhuis, Rose	77	Cory, Jenny S.	20, 107-STU, MC-5-STU, VI-1-STU
Burand, John P.	106-STU, DB-4, VI-14	Cossentine, Joan	22
Burge, Colleen A.	1	Cotes, Belén	FU-26
Burjanadze, Medea	19, MC-3	Coudron, T.A.	BA-3-STU
Byrne, Philip J.	67	Coutu, Cathy	149
		Crickmore, Neil	42, 61, BA-11-STU, MC-8
C		Cuartas, Paola	FU-19, VI-12, VI-13
Caballero, Primitivo	BA-16	Cuomo, Christina A.	38
Caetano, Isis A. L.	83-STU	Cusson, Michel	119
Cai, Jun	BA-15		
Cappelozza, Silvia	DB-5	D	
Carrière, Yves	44	D'Alessandro, Celeste P.	FU-2
Carrillo, J. Daniel	MC-12	Danforth, Bryan N.	DB-4
Carstens, Eric B.	37-STU, 54	Dara, Suchitra S.	FU-29-STU, MC-17-STU
Carvalho, Vanessa F. P.	MC-9, MC-10	Dara, Sumanth S. R.	FU-29-STU, MC-17-STU
Castagnola, Anaïs	88	Dara, Surendra K.	79, FU-29-STU, MC-17- STU, 130
Cavallo, E.C.	MC-14	De Bortoli, Caroline P.	MC-8
Çavdar, Sibel	34-STU	De Bortoli, Sergio A.	MC-8, MC-9, MC-10
Cave, Ronald D.	MC-12	De Fine Licht, Henrik H.	101
Chakroun, Maïssa	BA-16, BA-17	de Graaf, Dirk C.	49
Chateigner, Aurélien	118	Del Rincón-Castro, Ma. Cristina	BA-1-STU, BA-2-STU, VI- 9, 161
Chen, Ling	BA-21	Delalibera, Italo Junior	FU-2, FU-5-STU
Chen, Mean-Shine	121	Demarta, L.	82
Chen, Yue-hua	BA-15	Demirbag, Zihni	VI-23
Chen, Yun-Ru	70	Deng, Fei	55, 120
Chen, Yuting	73	Dennis, Jaclyn	16
Chen, Zhen	BA-19	Desidério, Janete A.	BA-9, BA-10
Chertkova, E.A.	MC-19		
Chikwana, Vimbai	BA-12		
Chkhubianishvili, Tsisia	NE-4-STU		

Desjardins, Christopher A.	38	Fast, Naomi M.	41-STU
Devine, Gregor J.	31	Faury, Nicole	155
Diabate, Abdoulaye	76	Federici, Brian A.	156, 157
Dias, Luciana P.	FU-7-STU	Fei, Zhangjun	70
Dillman, Adler	NE-7	Feng, Dongmei	BA-19
Dillon, Aoife B.	75	Feng, Ming-Guang	94, 95-STU, 96-STU, 97-STU, 98-STU
Dilpkumar, Aishwarya	NE-2	Ferguson, Colin	MC-2, 138
Djoumad, Abdelmadjid	119	Fernandes, Alesandra M.	FU-8
Do Nascimento, Ricardo M.	FU-2	Fernandes, Everton K.K.	FU-8, FU-14, FU-17-STU
Domanska, Barbara	BA-11-STU	Fernández-Navarro, Mariana	BA-1-STU
Dominic, Anto Raja	MI-2	Ferré, Juan	BA-16, BA-17
Donly, Cam	150	Ferreira, Paulo	FU-7-STU
dos Santos, Karine	FU-14	Ferreira, Rodrigo B.	FU-8
Downes, Sharon	10, BA-17	Fick, Will	FU-11
Drezen, Jen-Michel	119	Figueiredo, Camila S.	BA-9
Dubovskiy, I.M.	MC-19	Fisher, Joanna J.	136-STU
Dunlap, C.A.	BA-3-STU	Fleisher, Robert C.	48
Dunn, Alison	47	Floate, Kevin D.	BA-4-STU
E		Flor-Weiler, L.B.	BA-3-STU, MC-18
Ebeling, Julia	DB-2-STU	Folgarait, Patricia J.	24, FU-12, MC-14
Ebssa, Lemma	123	Fong, Ryan	16
Eigenbrod, Marina	37-STU	Fonseca, Fernando C. A.	15
Eilenberg, Jørgen	47, DB-5, FU-5-STU, 147	Foster, J.M.	92
Ekesi, Sunday	18, MC-13, 143	Foster, Jeremy	85-STU, 86-STU
El-Ayoubi, Mustapha	54	Foster, R. Nelson	FU-8
Elbashir, Mohammed I.	144	Franceshini, Sergio	28
Elkabir, Entisar	MI-1	Frank, Jacqueline A.	VI-10-STU
Elkinton, Joseph S.	106-STU	Franklin, Michelle T.	VI-4
Endo, Haruka	11, BA-14-STU	Franz, Alexander W. E.	30
Enkerli, Jürg	FU-5-STU, FU-23-STU	Freeman, Mark A.	6, 52
Ensi, Shao	64	Fritsch, Eva	105-STU, MC-4-STU
Erlandson, Martin	VI-4, VI-8, VI-15, 150, 151	Fünfhaus, Anne	DB-1-STU, DB-2-STU
Escriche, Baltasar	BA-16, BA-18	G	
Espinel, Carlos	78, MC-7	Galtier, Nicolas	117-STU, VI-5-STU
Etebari, Kayvan	30, 31	Gandelman, Olga	DB-3
Euerle, Chris	FU-4	Gao, Yulin	BA-5
F		Garcia, Lorena	MC-7
Fabrick, Jeffrey A.	44	Garcia-Gonzalez, Eva	DB-1-STU
Fang, Jim X. J.	BA-8, 158	Garcia-Suárez, Rosalina	BA-2-STU
Faria, Marcos	FU-6, MC-16	Gardescu, Sana	FU-22
		Garijalba, Erika	MC-7

Gaskin, John F.	FU-13	Guo, Shuyuan	BA-19
Gatsoquiannis, Christos	90	Guo, Suxia	BA-21
Gauthier, Debbie	FU-11, MI-3	Guo, Yajie	17
Gayral, Philippe	117-STU, VI-5-STU	Gürkan, M. Oktay	149
Gebhardt, Manuela	105-STU, VI-10-STU	Guzmán-Franco, Ariel W.	FU-9
Geden, C.	153	Gwak, Won Seok	DB-6-STU, DB-7, DB-8-STU, FU-10-STU, FU-28, VI-21-STU, VI-22-STU
Gelderman, Mandy	BA-7		25, 29
Genersch, Elke	DB-1-STU, DB-2-STU, MI-2, 154	Gwynn, Roma L.	
Ghiringhelli, Pablo D.	VI-7, VI-13		
Ghosh, Kaya	9		
Giraddi, R. S.	139	H	
Gisder, Sebastian	MI-2, 154	Haas, Jeffrey A.	46
Glare, Travis	25, 29, FU-27-STU, MC- 1, MC-15, 129-STU, 131- STU,	Hajek, Ann	83-STU, FU-22, 134, 136-STU
	MC-19	Hamajima, Rina	VI-17-STU
Glupov, V.V.	MC-19	Hamilton, Kristina	8
Gobinath, Chandrakasan	MC-20	Hampton, John	MC-15
Goble, Tarryn Anne	FU-22	Han, Bing	9
Goffré, Daniela	24, FU-12	Han, Yan	VI-4
Goldberg, Jonathan M.	38	Han, Yue	108-STU, 109
Golo, Patricia S.	FU-8	Hanson, M.A.	84-STU
Gómez, Demian	FU-20	Hapeshi, Alexia	93
Gómez, Juliana A.	VI-7	Harris, Stephanie	VI-15, 151
Gomis-Cebolla, Joaquin	BA-16	Harris, Sue E.	75
Gong, P.T.	BA-8, 158	Harrison, Robert L.	115, VI-8, VI-14
Gonzales, Yohandra	126	Hasler, James	45
González, Paula	FU-20	Hata, Toshimitsu	135
Gordon, Karl J. H.	10	Hazir, Selcuk	81, 122, NE-2
Grammare, Pierre M.	75	He, Kanglai	162, 163
Grant, Michelle	103	Healey, Joe	93
Graves, Leo	56, VI-16-STU	Heckel, David G.	10, 12, 32-STU
Greenwood, Spencer J.	67	Hegedus, Dwayne	34-STU, 149, VI-15, 150, 151
Griffin, Christine T.	3, 127	Heiman, David	38
Griffin, Samantha	BA-12	Henderson, Deborah	VI-4
Grijalba, Erika	78	Henkens, Marleen	114
Grisdale, Cameron J.	41-STU	Hernández-Dominguez, Carmela	FU-9
Grisi, Isabella	BA-6	Hernández-Martinez, Patricia	BA-16, BA-18
Grossi-de-Sa, Maria Fatima	15	Hernández-Rodriguez, Carmen S.	BA-16
Grøva, Lise	FU-23-STU	Hernio, Elizabeth A.	117-STU, 118, 119, VI-5-STU
Guan, Xiong	17, 64	Herrero, Salvador	14, 32-STU
Guan, Yi	98-STU		
Gueli Alletti, Gianpiero	37-STU, 69		
Guevara, Judith	MC-7		

Hesketh, Helen	47	Jaffuel, G.	NE-5
Hey, Timothy	BA-12	Jakka, Siva R. K.	45
Hice, Robert H.	156, 157	Jakubowska, Ágata K.	32-STU
Hicks, Ruth	51	James, Bill	BA-17
Hiltpold, I.	82, 124, NE-5	James, William J.	10
Holthe, Maren Pindsle	132	Jan, Eric	72
Holyoake, Andrew	131-STU	Janmaat, Alida F.	20, BA-7
Hoover, Kelli	VI-2	Jaronski, Stefan	FU-8, FU-13, 133
Hou, Dianhai	55, 120	Javed, Muhammad Afzal	VI-15
Hou, Jingtong	159	Je, Yeon Ho	BA-13-STU, VI-18
Hu, Zhihong	55, 120	Jech, Lerry E.	FU-8
Hua, Dong-hui	BA-15	Jehle, Johannes A.	37-STU, 69, 80-STU, 105-STU, MC-4-STU, VI-10-STU
Huang, Amy	VI-4	Jenkins, Nina E.	74
Huang, Fangneng	45	Jensen, Annette B.	DB-5
Huang, Ning	30	Jiang, Fan	163
Huang, Wei-Fone	7	Jiang, Kun	BA-15
Huang, Zhihong	60	Jin, Jie	FU-1
Hulse, Rosaline	68	Johnson, Dan	133
Humber, Richard A.	FU-14, FU-15, FU-16, FU-17-STU	Johnson, Karyn N.	35-STU
Hurst, Mark	63, MC-1, 138	Johnson, S.N.	82
Hussain, Mazhar	31	Jones, Sandra	138
		Jun, Young Soon	DB-6-STU, DB-7, DB-8-STU
I		Jurat-Fuentes, Juan Luis	45, 89
Ibarra, Jorge E.	BA-1-STU, BA-2-STU, 161, VI-9		
Ikeda, Motoko	VI-17-STU	K	
Imamura, Kazuhiro	BA-14-STU	Kabaluk, J. Todd	20, FU-25
Inan, Cihan	VI-23	Kakhadze, Manana	NE-4-STU
İnce, İkbál Agah	59	Kalmath, Basavaraj S.	139
Inglis, Susan	52	Kamalakaran, Siva	MC-20
Inoue, Maki N.	104-STU, VI-11-STU	Kapranas, Apostolos	127
Inouye, Kuniyo	BA-23	Kariithi, Henry M.	33-STU
Irons, Sarah	56, VI-16-STU	Karpinski, Anne	12
Ishii, Minehiro	FU-18-STU, FU-24-STU	Karthik Raja, Ramalingam	NE-2
Issi, Irma V.	MI-4	Katkar, Gajanan	139
Iwanicki, Natasha	FU-23-STU	Katsuma, Susumu	57-STU
Iwata, Kento	VI-11-STU	Kawchuk, Larry	133
Izumi Wilcoxon, Michi	16, 159	Kaya, Harry K.	122
		Keeling, Patrick J.	5
J		Kerr, Rose	51, 68
Jackson, Mark	81, FU-22	Keyser, Chad A.	101, 110, FU-8
Jackson, Trevor A.	MC-15, 140	Kikuta, Shingo	BA-20-STU

Kim, Jong Hoon	BA-13-STU, VI-18	Lemos, Manoel Victor F.	BA-9, BA-10
Kim, Woo Jin	BA-13-STU, VI-18	Leng, Song	16
Kim, Yonggyun	14, 36-STU, VI-24-STU	Leung, Joyce P. S.	20
King, Linda A.	56, VI-16-STU	Lewis, Edwin E.	125, 126, NE-1
Kleespies, Regina G.	37-STU, 47	Li, Ao	60
Klingen, Ingeborg	FU-23-STU, 132	Li, Leon Yu Zheng	VI-1-STU
Knispel, Henriette	DB-1-STU	Li, Lin	64
Kobayashi, Michihiro	VI-17-STU	Li, Xianchun	44
Koga, Hironori	BA-23	Li, Zhaofei	70
Koike, Masanori	FU-18-STU, FU-24-STU, NE-6-STU, 137-STU	Libro, Silvia	85-STU
Kokusho, Ryuhei	57-STU	Lightner, Don	88
Koppenhöfer, Albrecht M.	123	Li-Leger, Erica	FU-25
Kostromytska, Olga S.	123	Lima, Anabele A.	BA-6
Kraus, Bruna M.	MC-16	Lin, Jian	BA-21
Krejmer, Martyna	VI-3-STU, VI-6	Linde, Andreas	MI-2
Krell, Peter J.	53-STU, 58	Liu, Chenxi	BA-5
Kristmundsson, Árni	52	Liu, Sijun	64, 73
Kuhn, Lisa	119	Liu, Yang	53-STU, 58
Kumar, Sunil	36-STU, VI-24-STU	Liu, Zhaoxia	17
Kunimi, Yasuhisa	104-STU, 115, VI-11- STU	Loose, Jennifer S. M.	DB-1-STU
Kyei-Poku, George	FU-11, MI-3	Lopes, Rogário B.	MC-16, FU-6
		López-Tiajomulco, Juventino	VI-9
		Lovett, Brian	76-STU, 100-STU, FU-3-STU
L		Lu, Hsiao-Ling	99-STU, FU-4
Labrousse, Carole	118	Lucena, Wagner A.	15
Ladurner, Edith	28	Luck, Ashley N.	86-STU
Laengle, Tobias	145	Luke, Belinda	75
Lai, Qingying	60	Luo, Sijiani	55
Landmann, F.	92	Luo, Xiaoxia	BA-21
Lane, Thomas	89	Lupo, Sandra	FU-20
Lang, Alexander E.	90	Luttrell, Randall G.	BA-22
Larem, Andreas	MC-4-STU	Luz, Christian	FU-14, FU-15, FU-16, FU-17-STU
Lau, Sabina	16		
Laudier, Damien M.	VI-25	M	
Leclerque, Andreas	80-STU	MacDonald, Paul R.	107-STU
Lee, Seok Hee	BA-13-STU, VI-18	Macedo, Cristina	BA-6
Lee, Seung Hee	DB-6-STU, DB-7, DB-8- STU, FU-10-STU, FU-28, VI-21-STU, VI-22-STU	Madhani, Hiten D.	38
		Maguchi, Yohsuke	NE-6-STU
Lee, Siu Fai	10	Mahan, Rod J.	10
Leite, Luis G.	81	Maharramov, Jafar	49
Lemes, Ana Rita N.	BA-9	Maher, Abigail M.D.	127
Lemieux, Vincent	BA-12	Mahon, Rod	BA-17

Malania, Iatamze	NE-4-STU	Moore, Aubrey	140
Maniania, Nguya K.	18, MC-13, 143	Moore, Carmina	FU-8
Manker, Denise C.	27	Moore, Dave	75
Mansfield, Sarah	MC-1, MC-2, 138	Moreira, Helio	BA-6
Mantouvane, Fernando	FU-11	Morga, Benjamin	103
Marfetán, J.A.	MC-14	Mori, Gustavo Maruyama	FU-2
Marfetán, Jorge A.	FU-12	Morinerat, Rose	BA-6
Marion-Poll, Frederic	135	Mou, Yani	17
Marshall, Sean D.G.	MC-1, MC-2, 138, 140	Muermann, Martin	BA-7
Martin, Randy	148	Mulley, Geraldine	93
Martinez, Gonzalo	FU-20	Mumbi, Mercy	MC-13
Martins, Erica	BA-6	Munday, Peter	DB-3
Martins, Irene	FU-6	Muratoglu, Hacer	VI-23
Martins-de-Sa, Diogo	15	Murphy, Graeme	77
Marucci, Suzana C.	BA-10	Murugan, Kadarkarai	MC-20
Matiru, Vivienne	143	Mutune, Beritah	143
Maxwell, Danica	126	Myers, Judith H.	107-STU
Mayerhofer, Johanna	FU-5-STU		
McCaskill, David	BA-12	N	
McDonald, Mark	159	Nagoshi, Rodney	45
McGraw, Benjamin A.	123	Nagy, Éva	58
McKinnon, Aimee	131-STU	Nakai, Madoka	104-STU, 115, VI-11-STU
McMullen, John G. II	87-STU	Nakajima, Natsuko	BA-14-STU
Meagher, Robert	45	Nana, Paulin	MC-13
Meeus, Ivan	49, 50-STU	Nandalochana, Janitha B.	FU-8
Mejia, Cindy	78	Narva, Ken	BA-12
Meki, Irene K.	33-STU	Nelson, Mark	159
Mendoza, Artemio Mendoza	131-STU	Nelson, Tracey	MC-1
Meusch, Dominic	90	Neunemann, David	32-STU
Meyling, Nicolai V.	26, 101, FU-5-STU, FU-23-STU, FU-26	Niassy, Saliou	143
Michalski, M.L.	92	Nicolson, Laurel	FU-8
Mikaia, Nona	NE-3	Nielsen-LeRoux, Christina	DB-5
Millard, Andrew	93	Niu, Jinzhi	50-STU
Mitra, Alok	63	Norli, Hans R.	FU-26
Mizuki, Eiichi	BA-23	Nunn, Adam J.	75
Moffitt, Jessica	30		
Moldovan, Anna I.	MC-6-STU	O	
Molotievskiy, Natalia	MC-6-STU	O'Callaghan, Kate	127
Munteanu		O'Callaghan, Maureen	MC-1, 138
Monnheimer, Laurin R.	69	Oikawa, Fuka	FU-24-STU
Montalva, Cristian	FU-14, FU-15, FU-16	Okumura, Shiro	BA-23
Monterroso, Armando	MC-12	Oneal, Meghan	160

Ono, Chikako	VI-19-STU	Queiroz, Paulo	BA-6
Oppert, Cris	89	Quitugua, Roland J.	140
Orozco, Rousel	62-STU, 88	R	
Ozdemir, Esengul	34-STU, 149	Raad, Maya	129-STU
P		Rabalski, Lukasz	VI-3-STU, VI-6
Pan, Kai	120	Rämert, Birgitta	FU-26
Park, Hyun-Woo	156, 157	Rangel, Drauzio E. N.	FU-7-STU
Park, Youngjin	14	Rännbäck, Linda-Marie	FU-26
Parmentier, Laurian	49	Raunser, Stefan	90
Passarelli, A. Lorena	30	Raymond, Ben	4, 128
Patel, Anant	21, 111	Raza, Fahreen	56
Patil, B.V.	139	Reddy, Gadi V. P.	FU-13
Pauchet, Yannick	12	Rehner, Stephen	FU-13
Pelegrini, Patricia B.	15	Reineke, Annette	10
Pelz, Juliana	19	Reinoso-Pozo, Yaritza	161
Peng, Donghai	BA-21	Renault, Tristan	155
Pereira, Roberto M.	23	Richard, Stefan	VI-4
Perera, Omaththage P.	89, 116, BA-22	Richtman, Nina	160
Pérez-Martinez, José M.	MC-12	Ridgway, Hayley	131-STU
Periquet, Georges	119	Rivas, Federico	FU-20, MC-15
Perlman, Steve J.	2, 84-STU	Rivera, Hugo Fernando	MC-7
Perumal, Pachiappan	NE-2	Rizhamadze, Irine	NE-4-STU
Philippi, Jasmin	80-STU	Roberts, Donald W.	110, FU-8
Pijlman, Gorben P.	59	Robertson, Mairi	22
Piot, Niels	49	Rocha, Luiz F. N.	FU-14
Polanczyk, Ricardo A.	BA-9, MC-8	Rodriques de Castro, Thiago	FU-5-STU
Pond, Michelle	DB-3	Rodriques, Juscelino	FU-17-STU
Ponnuraj, Jeyakumar	44	Romagnoi, E.M.	BA-3-STU
Pontieve, Cindy	118	Rooney, A.P.	BA-3-STU
Poppinga, Lena	DB-1-STU, DB-2-STU	Rooney, Alejandro P.	MC-18
Possee, Robert D.	56, VI-16-STU	Ros, Vera I.D.	108-STU, 109
Potin, Olivier	75	Ross, Stuart	51
Potvin, Léna	BA-12	Rostas, Michael	FU-27-STU, MC-15, 129-STU
Prabhuraj, A.	139	Roux, Louise-Marie D.	67
Praça, Lilian	BA-6	Roy, Helen	47
Pupin, Breno	FU-7-STU	Royalty, Reed N.	27
Purse, Beth	47	Ruan, Lifang	BA-21
Pushparajan, Charlotte	141	Ruiz de Escudero, Inigo	BA-16
		Ruiz, Carolina	78
		Ruiz, Gregory M.	48
		Ruiz-Vega, J.	NE-1
		Rusakovich, Georgiy I.	39
Q			
Qi, Jiaheling	137-STU		

Rybakova, Daria	63	Simeto, Sofia	FU-20
S		Sitaram, Anand	91
Saar, Katharina	80-STU	Sivaramakrishnan, Sivaperumal	NE-2
Sahoo, Alap	FU-29-STU, MC-17-STU	Skhirtladze, Rusudan	NE-4-STU
Saito, Taro	77	Skrzecz, Iwona	VI-3-STU, VI-6
Saito, Yasumasa	104-STU	Slatko, Barton	85-STU, 86-STU, 92
Sanscrainte, Neil	38	Smagghe, Guy	49, 50-STU
Santos, Adriana	78	Snodgrass, Gordon L.	BA-22
Santos, Eduarda M.	65-STU, 66	Soares, C. Marcelo	BA-6
Sato, Masanao	VI-19-STU, VI-20-STU	Soberon, Mario	BA-6
Sato, Ryoichi	11, BA-14-STU, BA-20-STU	Sokolova, Yuliya Y.	39, MI-4
Sauer, Annette J.	105-STU	Solter, Leellen	7, 106-STU
Schäfer, Johannes	19	Song, Fuping	61
Schal, Coby	74	Sosa-Gómez, Daniel R.	MC-5-STU, 142
Schijlen, Elio	114	Sotelo, Paula	FU-19
Schjøll, Annette Folkedal	FU-23-STU	Souza, Daniela A.	FU-6
Schliephake, Edgar	80-STU	Srikumar, A.	82
Schneider, Diana	VI-10-STU	St. Leger, Raymond	76, 99-STU, 100-STU, 113, FU-3-STU, FU-4
Schumann, Mario	21	Stark, JoAnn	FU-8
Schwartz, Hillel	NE-7	Staton, Margaret	89
Schwartz, Jean-Louis	BA-12	Steinwender, Bernhardt M.	101
Scoates, Freya C. L.	75	Stentiford, Grant D.	40-STU, 47, 51, 65-STU, 66, 68, DB-3
Sebastião, Isis	BA-9	Stephan, Dietrich	19, 80-STU
Seib, Christopher	19	Sternberg, Paul	NE-7
Selamet, Stefanie	FU-8	Stevanovic, Aleksej L.	35-STU
Senderskiy, Igor V.	39	Stevens, Tristan	13
Shajahan, Johnny	FU-11	Stewart, Don	119
Shang, Yu	120	Stock, Patricia	62-STU, 87-STU, 88
Shankarganesh, K	144	Stoltz, Don	119
Shapiro-Ilan, David	81, 125, 126, 128, 122	Storm, Clare G.	75
Sharma, P.	144	Strand, Michael R.	71
Sherwood, Samuel P.	FU-8	Strasser, Hermann	FU-23-STU
Shi, Liangen	FU-1	Strickman, Daniel A.	MC-18
Shikano, Ikkei	VI-2	Suisse, Holly C.	FU-8
Shimada, Toru	57-STU	Sullivan, W.	92
Shin, Tae Young	DB-6-STU, DB-7, DB-8-STU, FU-10-STU, FU-28, VI-21-STU, VI-22-STU	Sun, Ming	BA-21
Shu, Changlong	61	Supatasvili, Archil	MC-3
Sieminska, Edyta	151	Suthaparan, Aruppillai	132
Silva, Joseane	BA-6	Swaminathan, Jayanthi	MC-15, 138
		Szeqczyk, Boguslaw	VI-3-STU, VI-6

T

Tabashnik, Bruce E.	44
Tabert, Matilda	VI-4
Taka, Hitomi	VI-19-STU
Tanaka, Shiho	11, BA-20-STU
Tang, Li	96-STU
Tao, Xue Ying	VI-18
Tay, Wee Tek	10
Taylor, Bryony	75
Taylor, Nick	51
Teng, Chao-Yi	121
Teng-Nelson, Kathy	88
Theilmann, David A.	53-STU, VI-8, VI-15, 150, 151
Thomas, Matthew B.	74
Thompson, Stephan	160
Toderas, Ion K.	MC-6-STU
Tokarev, Yuri S.	39, MI-4
Toprak, Umut	34-STU, 149
Torchin, Mark E.	48
Torp, Torfinn	132
Townsend, Richard	MC-2, 138
Treat, Scott	FU-8
Turlings, T.C.J.	NE-5
Tyler, Charles R.	65-STU, 66

U

Uchida, Haruaki	115
Uda, Momoka	VI-20-STU
Undorf-Spahn, Karin	MC-4-STU, 105-STU

V

Vaaje-Kolstad, Gustav	DB-1-STU
Vacari, Alessandra M.	MC-8, MC-9, MC-10
Vachon, Vincent	BA-12
van Aerle, Ronny	65-STU, 66
van der Werf, Wopke	114
van Houte, Stineke	108-STU, 109
van Oers, Monique M.	33-STU, 47, 59, 108- STU, 109
Vaquolo, Maclean	140
Vera-Velasco, Natalia M.	BA-16, BA-18
Verbruggen, Bas	65-STU, 66

Verdejo, Eva Fortea	BA-12
Vidal, Stefan	21, 111
Villamizar, Laura	FU-19, VI-7, VI-12, VI-13
Visnovsky, Gabriel	141
Vlak, Just M.	33-STU, 59, 114, DB-5,
Vogel, Heiko	32 - STU
Volkoff, Anne-Nathalie	119

W

Wadowska, Dorota W.	67
Wakefield, Maureen E.	75
Walsh, Thomas K.	10, BA-17
Wang, Ding-Yi	98-STU
Wang, Hualin	55, 120
Wang, Jonathan B.	99-STU, FU-4
Wang, Manli	55, 120
Wang, Ping	43, 70
Wang, Ranran	55
Wang, Tingting	BA-15
Wang, Yafang	17
Wang, Yidong	162
Wang, Yueqin	162
Wang, Zhenying	162, 163
Wang, Zhi-kang	95-STU
Ward, Georgia	68
Wasag, Bartosz	VI-6
Waterfield, Nick	93
Wei, Y.J.	158
Weill, Mylène	VI-5-STU
Weis, Michael	150
Weiss, Louis M.	9
Wennmann, Jörg T.	37-STU, 69, VI-10-STU
Werner, Manuel	80-STU
Wessman, Per	MC-15
West, Michelle	BA-11-STU
Westrum, Karin	FU-23-STU, 132
Whelan, Thomas A.	41-STU
Wiebe, Craig	133
Wilches, Diana	BA-4-STU
Williams, Adam K.	10
Williams, Bryony A. P.	8, 40-STU
Williams, Thomas	40-STU

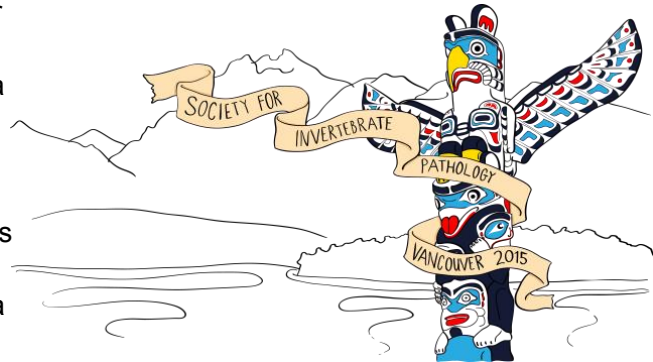
Willis, Leslie G.	150	Zenner, Annemie	127
Wilson, Derick	138	Zhang, Chunlu	BA-19
Wilson, Michael	MC-1, 138	Zhang, Feiping	17
Wiredu-Boakye, Dominic	40-STU	Zhang, Jie	61
Wirth, Margaret C.	156	Zhang, Lingling	17
Wisch, Luca N.	MC-5-STU	Zhang, Long-Bin	96-STU
Woo, Soo Dong	DB-6-STU, DB-7, DB-8-STU, FU-10-STU, FU-28, VI-21-STU, VI-22-STU	Zhang, Nan	BA-19
Wright, David	138	Zhang, Ni	BA-21
Wright, Glenda M.	67	Zhang, Tiantao	163
Wu, Kongming	BA-5	Zhang, W. F.	BA-8
Wu, Shaohui	123, FU-13	Zhang, Xuewen	61
Wu, Songging	17	Zhang, Y.	158
Wu, Tzongyuan	121	Zhang, Yanfang	55
Wu, Yidong	BA-5	Zhang, Zhe	133
		Zhao, Haizhou	60
		Zhao, Jian-Zhou	160
		Zheng, Jinshui	BA-21
		Zheng, Shuning	DB-4
		Zheng, Yi	159
		Zhong, Silin	70
		Zhou, Fengqiao	55
		Zhou, Y.	BA-8, 158
		Zhu, Jing	97-STU
		Zhu, Xiaoli	17
		Ziemnicka, Jadwiga	VI-3-STU
		Zou, Shuangquan	17
		Zucchi, Maria I.	FU-2
		Zydenbos, Sue	MC-1
X			
Xiao, Gengfu	120		
Xiao, Yutao	MC-11-STU		
Xie, Weiping	16		
Xiong, Yueting	17		
Xu, Xiaoping	BA-12		
Xu, Zhuoying	17		
Y			
Yamamoto, Takashi	16, 159		
Yanagawa, Aya	135		
Yang, Kai	60		
Yaroslavtseva, O.N.	MC-19		
Yin, Yunqiang	91		
Ying, Pang	BA-13-STU, VI-18		
Ying, Sheng-Hua	94		
Yoshiga, Toyoji	NE-6-STU		
Yoshimura, Tsuyoshi	135		
You, You	16		
Young, Sarah	38		
Yuan, Meijin	60		
Yuan, Yu	BA-15		
Z			
Zeng, Qiandong	38		

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The SIP 2015 Meetings Logo was created by Laura Ulrich, a lover of both art and science and a Simon Fraser University student in Burnaby British Columbia. Having almost completed her B.Sc. in Biological Sciences, she is planning on attending the University of Toronto in Mississauga to study in the Biomedical Communications graduate program. Her blog, Monsters and Molecules (<https://monstersandmolecules.wordpress.com>), is a crossroads between art and science as she shares her journey to become a professional scientific illustrator. The logo was inspired by the Canadian west coast's geographical splendor and First Nations cultural heritage. The totem pole is based on one of the nine found in Vancouver's Stanley Park, telling a story through the characters they depict.



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