SIP
SOCIETY FOR INVERTEBRATE PATHOLOGY

Tours 2016

49th Annual Meeting
of the
Society for Invertebrate Pathology

International Congress
on Invertebrate Pathology and Microbial Control

24-28th of July 2016
Vinci Centre International de Congrès
Tours - France

Program at a Glance
SIP 2016 – 24-29 / 07/2016 -Tours - France

Unless specified, all events will be located at the Vinci International Conference Centre

Sunday – July 24

08:30-17:30	Registration	*Hall*
09:00-17:00	Council Meeting	*Montlouis*
14:00-17:00	**Bacteria Division Workshop**	*Chinon*
	'Bt Nomenclature'	
18:00-21:30	**Mixer - Hotel de Ville de Tours**	

Monday – July 25

07:30-08:15	*Qi-Gong*	*Foyer*
08:00-17:00	Registration	*Hall*
08:30-09:00	**Opening Ceremony**	*Descartes*
09:00-10:00	**Founders Lecture**	*Descartes*
10:00-10:30	*Coffee Break*	*A. Sorel*
10:30-12:30	**Plenary Symposium**	*Descartes*
	'Insect for Food and Feed'	
12:30-14:00	*Buffet Lunch*	*A Sorel*
	Junior Researcher Workshop	*Chinon*
	'Postdoctoral funding opportunities in the EU & US'	
	JIP Editorial Board Meeting	*Montlouis*
14:00-16:00	**Symposium Dis. of Beneficial Invertebrates**	*Courteline*
	'Mollusc Diseases'	
	Special EU Cost Action session	*Descartes*
	"Ménage à trois - Three way interactions between plants, arthropods and microbes that benefit the plants"	
	Session Virus 1	*Vouvray*
	Session Bacteria 1	*Chinon*
16:00-16:30	*Coffee Break*	*A Sorel*
16:30-18:30	**Symposium Fungi Division**	*Descartes*
	'How fungi mediate protection against herbivores and plant pathogens'	
	Session Disease of Beneficial Invertebrates 1	*Courteline*
	Session Virus 2	*Vouvray*
	Session Microbial Control 1	*Chinon*
18:30-19:45	Division Business Meetings and workshops:	
	Microsporidia Workshop: *'Hot topics in microsporidia research'*	*Courteline*
	Bacteria	*Vouvray*
	Fungi	*Chinon*

Tuesday – July 26

07:00-07:45	*Qi-Gong*	*Foyer*
07:35-12:30	Registration	*Hall*
08:00-10:00	**Special Symposium**	*Vouvray*
	'Human impact on pathogens-honeybee interactions'	
	Cross Division Symposium	*Chinon*
	'Recruitment of beneficial microbes and nematodes'	
	Session Bacteria 2	*Courteline*
	Session Fungi 1	*Bourgueil*
10:00-10:30	*Coffee Break*	*A Sorel*
10:30-12:30	**Symposium Microbial Control Division**	*Chinon*
	'Next Generation Biopesticides'	
	Session Virus 3	*Vouvray*
	Session Microsporidia	*Bourgueil*
	Session Bacteria 3	*Courteline*
12:30-17:30	*Optional Excursion to Amboise*	
16:30-22:00	**Garden Party – Domaine de Candé (BBQ & Race)**	

Wednesday – July 27

07:30-08:15	*Qi-Gong*	*Foyer*
08:00-16:00	Registration	*Hall*
08:30-10:30	**Cross Division Symposium**	*Chinon*
	'Next Generation Sequencing'	
09:00-10:30	Session Nematodes	*Bourgueil*
08:45-10:30	Session Virus 4	*Courteline*
08:30-10:30	Session Microbial Control 2	*Vouvray*
10:30-11:00	*Coffee Break*	*A Sorel*
10:30-13:00	**Poster Session**	*A Sorel*
12:00-13:30	*Cocktail Lunch*	*A Sorel*
12:30-13:30	ICTV Meeting	*Bourgueil*
13:30-15:30	**Symposium Bacteria Division**	*Chinon*
	'Unity and diversity of Entomopathogenic bacteria'	
	Session Fungi 2	*Bourgueil*
	Session Virus 5	*Vouvray*
	Session Microbial Control 3	*Courteline*
15:30-16:00	*Coffee Break*	
16:00-18:00	**Symposium Nematode Division**	*Chinon*
	'Harnessing Metabolites from Entomopathogenic Nematode Symbiotic Bacteria for Broad Use'	
	Session Microbial Control 4	*Courteline*
	Session Disease of Beneficial Invertebrates 2	*Bourgueil*
	Session Virus 6	*Vouvray*
18:00-19:45	Division Business Meetings and Workshops:	
	Microbial Control	*Courteline*
	Nematodes Workshop: *'Shooting a Worm'*	*Chinon*
	Virus Workshop: *'Taxonomy of Polydnaviridae'*	*Vouvray*
	DBI Workshop: *'Coral Diseases'*	*Bourgueil*
18:00-20:00	**Outreach at the Guingette**	

Thursday – July 28

07:00-07:45	*Qi-Gong*	*Foyer*
08:00-14:00	Registration	*Hall*
08:00-10:00	**Symposium Virus Division**	*Descartes*
	'Viruses and horizontal gene transfers'	
	Session Fungi 3	*Courteline*
	Session Disease of Beneficial Invertebrates 3	*Bourgueil*
	Session Microbial Control 5	*Vouvray*
10:00-10:30	*Coffee Break*	
10:30-12:30	**SIP Business Meeting**	*Descartes*
12:30-14:00	*Buffet Lunch*	*A Sorel*
12:30-14:00	Student Competition Jury	*Vouvray*
14:00-16:00	**Symposium Microsporidia Division**	*Bourgueil*
	'Host Pathogen interactions'	
	Session Bacteria 4	*Courteline*
	Session Virus 7	*Descartes*
	Session Microbial Control 6	*Vouvray*
16:00-16:30	Student Business Meeting	*Vouvray*
18:00-01:00	**Banquet – Grange de Meslay**	

Friday – July 29

Location: IRBI UMR CNRS 7261, Université François Rabelais

09:00-17:00	**Summer School on Invertebrate Pathology**	
	Optional	

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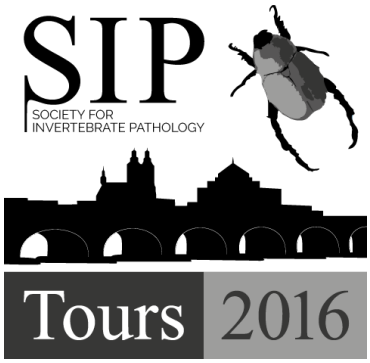

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2016 PROGRAM

Important Notes

Attendants shall <u>not take pictures</u> from projections during presentations

The abstract included in this book should not be cited in print without the author's permission

STU indicates STUDENT presentation
000 indicates the number of ORAL presentation
BA – 00 indicates abstract number for POSTER presentation

SUNDAY – 24 July

09:00-17:00 **SIP Council Meeting** *Montlouis*
10:00-17:30 Registration *Hall*

BACTERIA WORKSHOP 14:00-17:00 - *Chinon*

The future of the Bt nomenclature

Niel Crickmore & Colin Berry

18:00-21:30 **Mixer** Welcome in Tours by A.Godbert *Hotel de Ville*

MONDAY – 25 July

07:30-08:30 **Qi Gong** *Foyer*
08:00-17:00 **Registration** *Hall*

08.30-10:00 - *Descartes*

Opening Ceremony and SIP Founders' Lecture

08:30 **Opening Ceremony** *Descartes*

Welcome Address

Philippe Roingeard Vice-President of the University Union "Léonard De Vinci"
Elisabeth Herniou, Chair, Organising Committee
Peter Krell, President of the SIP

Award Presentations

Monique van Oers, Chair, Awards & Student Committee

Founders' Memorial Lecture

James Becnel, Chair, Founders' Lecture Committee
Honoree: David Ellar
Lecturer: Nell Crickmore

10:00-10:30 **COFFEE BREAK** *Agnès Sorel*

PLENARY SYMPOSIUM 10:30-12:30 - *Descartes*

Insects for Food and Feed

Christina Nielsen Leroux

10:30 **1** **Opportunities and Constraints of farming Insects for food and feed: a global review** - Paul Vantomme, UN Food and Agriculture Organization (FAO), Rome, Italy

11:00 **2** **Industrialization of Insect Farming: New challenges to prevent pathogenic hazards** - Thomas Lefebvre, YNSECT, Genopole, Evry, France

11:30 **3** **Managing insect viruses in insect factories for food and feed: Successful management of an insect virus, *Glossina pallidipes* salivary gland hypertrophy virus, from an insect factory -** Adly Abdalla, Insect Pest Control Laboratory, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, IAEA, Vienna, Austria

12:00 **4** **Pathogenic aspects in insects produced for feed and food** - Jørgen Eilenberg, Department of Plant and Environmental Sciences, University of Copenhagen, Denmark

12:30-14:00 **BUFFET LUNCH** *Agnès Sorel*

12:30-14:00 JIP Editorial Board Meeting *Montlouis*

JUNIOR RESEARCHER WORKSHOP 12:30-14:00 - *Chinon*

Postdoctoral funding opportunities in the EU & US

Postdoctoral funding opportunities in the EU
Allyre Lohier, DR8, CNRS, Orléans, France
Postdoctoral funding opportunities in the US
Rollie Clem, BIO/IOS, NSF, Arlington, United States

SYMPOSIUM DISEASES OF BENEFICIAL INVERTEBRATES 14:00-16:00 - *Courteline*

Mollusc diseases

Grant Stentiford

14:00 **5** **New perspective on the microcell parasites** - Isabelle Arzul, IFREMER, Station de La Tremblade, France

14:30 **6** **A new phylogeny and eDNA insight into paramyxids: an increasingly important but enigmatic clade of protistan parasites of marine invertebrates** - Georgia Ward[1], Martyn Bennett[2,3], Kelly Bateman[2], Grant Stentiford[2], Rose Kerr[2], Stephen Feist[2], Suzanne Williams[1] Cedric Berney[1], David Bass[1,2,]; [1]Natural History Museum, London, United Kingdom; [2]CEFAS, Weymouth, United Kingdom; [3]School of Biosciences, Univ. of Exeter, United Kingdom

15:00 **7** **Viral diseases affecting marine bivalves** - Tristan Renault, IFREMER Nantes, Université de Nantes, France

15:30 **8** **Breeding for disease resistance: Development of a *Crassostrea gigas* SNP array -** Tim Bean[1], Alejandro Gutierrez[2,3], Richard Paley[1], Chantelle Hooper[1], Matthew Sanders[1], Craig Stenton[1], Karim Gharbi[3], Ross Houston[2,3], [1]Centre for Environment, Fisheries and Aquaculture Science, Weymouth, United Kingdom; [2]The Roslin Institute and R(D)SVS University of Edinburgh, United Kingdom; [3]Edinburgh Genomics, University of Edinburgh, United Kingdom

EU COST Action FA1405 SESSION 14:00-16:00 - *Descartes*

***Ménage à trois*:**

Three way interactions between plants, arthropods and microbes that benefit the plants

Richard Meadow & Maria Pozo

14:00 **9** **A fungal endophyte helps plants to tolerate root herbivory through changes in gibberellin and jasmonate signalling -** Marco Cosme[1], Jing Lu[2], Matthias Erb[3], Michael Stout[4], Philipp Franken[5], Susanne Wurst[6], [1]Plant–Microbe Interactions, Department of Biology, Utrecht University, Utrecht, Netherlands; [2]Institute of Insect Science, Zhejiang University, Hangzhou, China; [3]Institute of Plant Sciences, University of Bern, Switzerland; [4]Department of Entomology, Louisiana State University Agricultural Center, Baton Rouge, United States; [5]Department of Plant Propagation, Leibniz; Institute of Vegetable and Ornamental Crops, Erfurt-Kuehnhausen, Germany; 6Functional Biodiversity, Dahlem Center of Plant Sciences, Institute of Biology, Freie Universitat Berlin, Germany

14:15 **10** **Uncovering the effects of cover crops and soil characteristics on *Metarhizium*-plant-insect interactions in an organic cropping system** - Puneet Randhawa[1], Imtiaz Ahmad[2], Dawn Luthe[1], Mary Barbercheck[1], [1]Pennsylvania State University, United States; [2]Quaid-i-Azam University, Islamabad, Pakistan

14:30 **11-STU** **Induced plant defense accomplished by a grass endophyte** - Benjamin Fuchs, Jochen Krauss, Department of Animal Ecology and Tropical Biology, Biocenter, University of Würzburg, Germany

14:45 **12** **Systemic grass endophytes and their importance for herbivores in Europe** - Jochen Krauss, Department of Animal Ecology and Tropical Biology, Biocenter, University of Würzburg, Germany

15:00 **13** **Plant metabolic responses to endophytic colonization by *Trichoderma* and *Epichloe* and their effect on insects** - Michael Rostas, Daniel Maag, Diwakar Kandula, Mike Cripps, Caroline Mueller, Patrick Silcock. Bio-Protection Research Centre, Lincoln University, New Zealand

15:15 **14** **Endophytic entomopathogenic *Metarhizium brunneum* against insect pests: novel integrated fermentation and formulation strategies** - Anant Patel[1], Stefan Vidal[2], Laurenz Hettlage[1], Desiree Jakobs-Schoenwandt[1], Vivien Krell[1]; [1]Bielefeld University of Applied Sciences, Department of Engineering Sciences and Mathematics, Bielefeld, Germany; [2]Göttingen Department of Crop Sciences, University of Göttingen, Göttingen, Germany

15:30 **15** **Determination of destruxin A in potato plants after foliar spray of *Metarhizium brunneum*** - Alex Rios-Moreno, Inmaculada Garrido-Jurado, Gloria Resquin-Romero, Lourdes Arce, Enrique Quesada Moraga, University of Córdoba, Spain

CONTRIBUTED PAPERS 14:00-16:00 - *Vouvray*

Virus 1

David Theilmann & Kai Yang

14:00 **16-STU** **Protein tyrosine phosphatase 2 from the baculovirus SeMNPV induces apoptosis in insect cells -** Yue Han, Stineke Van Houte, Susan Van Aalst, Monique Van Oers, Vera Ros, Laboratory of Virology, Wageningen University, Netherlands

14:15 **17-ST** **Characterization of AcMNPV encoded viral ubiquitin and its association with AC141 for the production of budded virus -** Siddhartha Biswas[1], Leslie Willis[2], David Theilmann[1,2,] [1]University of British Columbia, Vancouver, Canada; [2]Summerland Research and Development Centre, Summerland, British Columbia, Canada

14:30 **18-STU** **3-Dimensional ultrastructural modelling of**

Autographa californica multicapsid nucleopolyhedrovirus infection in insect cells to determine the role of P10 during baculovirus infection - Leo Graves[1], Louise Hughes[1], Sarah Irons[1], Linda King[1], Possee Robert[1,2]; [1]Oxford Brookes University, Headington Campus, Oxford, United Kingdom; [2]Oxford expression technologies, United Kingdom

14:45 **19** **The Autographa californica multiple nucleopolyhedrovirus ac54 gene is crucial for the localization of the major capsid protein VP39 at the site of nucleocapsid assembly -** Zhanwen Guan, Ling Zhong, Chunyan Li, Wenbi Wu, Meijin Yuan, Kai Yang**,** State Key Laboratory of Biocontrol, Sun Yat-sen University, Guangzhou, China

15:00 **20** **Structural and functional analyses of the sulfhydryl oxidase P33 of Autographa californica multiple nucleopolyhedrovirus -** Kuang Wenhua, Hou Dianhai, Zhang Huanyu, Wang Manli, Zhou Ningyi, Deng Fei, Wang Hualin, Gong Peng, Zhihong Hu; State Key laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, China

15:15 **21** **Functional analysis of the conserved cysteines of AcMNPV GP41 -** Li Yimeng, Wang Manli, Shen Shu, Hu Liangbo, Hu Zhihong, Deng Fei, Wang Hualin; State Key Laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, China

15:30 **22** **Autographa californica multiple nucleopolyhedrovirus (AcMNPV) PIF protein AC83 is required for nucleocapsid assembly for both ODV and BV as well as recruitment of the PIF complex to the ODV envelopes -** Muhammad Javed[1], Leslie Willis[2], Stephanie Harris[1], Martin Erlandson[1], B. Cam Donly[3], Dwayne Hegedus[1], Monique Van Oers[4], David Theilmann[2]; [1]Saskatoon Research and Development Centre, Agriculture and Agri-Food Canada, Saskatoon, Canada; [2]Summerland Research and Development Centre Agriculture and Agri-Food Canada, Summerland, Canada; [3]London Research and Development Centre Agriculture and Agri-Food Canada, London, Canada; [4] Laboratory of Virology, Wageningen University Wageningen, Netherlands

15:45 **23** **The host specificities of baculovirus *per os* infectivity factors** - Song Jingjiao[1,2], Wang Xi[1], Huang Huachao[1], Deng Fei[1], Wang Hualin[1], Arif Basil[3], Hu Zhihong[1], Manli Wang[1]; [1]State Key Laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, China; [2]Experimental medicine center, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China; [3]Laboratory for Molecular Virology, Great Lakes Forestry Centre, Sault Ste. Marie, Ontario, Canada

CONTRIBUTED PAPERS 14:00-16:00 - *Chinon*

Bacteria 1

Ken Narva & Peter Kupferschmied

14:00 **24** **Immunomodulatory activity of *Brevibacillus laterosporus* on the house fly** - Maria Elena Mura, Luca Ruiu, Department of Agriculture, University of Sassari – Italy; and Bioecopest Srl – Technology Park of Sardinia, Tramariglio, Italy

14:15 **25** **Immune and detoxification systems of Colorado potato beetle infected with bacteria *Bacillus thuringiensis* -** Olga Polenogova, Ekaterina Grizanova, Olga Yaroslavtseva, Viktor Khodyrev, Ivan Dubovskiy**;** Institute of Systematics and Ecology of Animals, Siberian Branch of Russian Academy of Science, Russia

14:30 **26 -STU** **Immune priming might have evolved from infection by Gram+ bacterial pathogens in the mealworm beetle**, ***Tenebrio molitor***. - Julien Dhinaut, Manon Chogne, Yannick Moret; Biogéosciences, Université de Bourgogne, CNRS UMR6282 Dijon, France

14:45 **27-STU** **Identification of synergist interactions between midgut bacteria of *Lymantria dispar* larvae and *Bacillus thuringiensis* HD-1 -** Zane Metla[1], Monika Maurhofer[2], Liga Jankevica; [1]Laboratory of Experimental Entomology and Microbiology, Institute of Biology, University of Latvia, Latvia; [2]ETH, Zurich, Switzerland

15:00 **28-STU** **Plasmid-borne rap-phr systems control sporulation of *Bacillus thuringiensis* in insect larvae** - Fernanda Fazion[1,2], Stéphane Perchat[1], Christophe Buisson1, Gislayne Vilas-Boas[2], Didier Lereclus[1]; [1]INRA Micalis Institute, INRA, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France; [2]Universidade Estadual de Londrina, Brazil

15:15 **29-STU** **A bioassay method to determine the insecticidal activity of Bacillus thuringiensis against *Ceratitis capitata* (Diptera: Tephritidae) and *Drosophila suzukii* (Diptera: Drosophilidae**) - Daniel Valtierra[1,2], Isabel Matas[1], Javier Caballero1,2, Primitivo Caballero[1,2]; [1]Instituto de Agrobiotecnología, CSIC-UPNA, Spain; [2]Depart. de Producción Agraria, Universidad Pública de Navarra, Pamplona, Spain

15:30 **30-STU** **Susceptibility of *Grapholita molesta* (Busck, 1916) to *Bacillus thuringiensis*, individual toxins and their mixtures** - Joaquin Gomis-Cebolla[1], Ana P. S Ricietto[1,2], Gislayne T Vilas-Bôas[2], Juan Ferré[1]; [1]University of València, ERI of Biotechnology and Biomedicine, Departamento de Genética, Facultad de Ciencias Biológicas, Burjassot, Spain; [2]Centro de Ciências Biológicas Universidade Estadual de Londrina, Brazil

15:45 **31-STU** **Interactions between HepG2 and Parasporin-3** - Wided Souiss, Samuel Colosimo, Tweedie Alistair, Michelle West, Neil Crickmore; Department of Biochemistry, School of Life Sciences University of Sussex, Falmer, Brighton, United Kingdom

16:00-16:30 **COFFEE BREAK** *Agnès Sorel*

SYMPOSIUM OF THE FUNGI DIVISION 16:30-18:30 - ***Descartes***

How Fungi mediate protection against herbivores and plant pathogens

Nicolai Vitt Meyling & Maya Raad

16:30 **32** **Plant protection potential of entomopathogenic fungi as endophytes: What is the evidence and what is the mechanism?** - Nicolai Meyling[1], Aimee Mckinnon[2], Maya Raad[2], Maria Moran-Diez[2], Travis Glare[2], Susanna Saari[1]; [1]Department of Plant and Environmental Sciences, University of Copenhagen, Denmark; [2]Bio-Protection Research Centre, Lincoln University, New Zealand

16:45 **33** Microbial-induced resistance against herbivores: mechanisms and ecological consequences - Ana Pineda; Netherlands Institute of Ecology, NIOO-KNAW, Netherlands

17:10 **34** **Priming of plant defenses against herbivores by arbuscular mycorrhizal fungi** - Maria J. Pozo[1], Javier Rivero[1], Javier Lidoy[1], Victor Flors[2]; [1]Estacion Experimental del Zaidin, CSIC, Granada, Spain; [2]Universitat Jaume, Castello de la Plana, Spain

17:35 **35** **Induced systemic resistance by *Trichoderma spp* -** Christine Vos[1,2,3], Katrijn Raymaekers[1,2], Yuxia Yang[1], Kaat De Cremer[1,2], Barbara De Coninck[1,2], Kemal Kazan[3], Bruno Cammue1[2]; [1]KU Leuven Centre of Microbial and Plant Genetics, Leuven, Belgium; [2]VIB Department of Plant Systems Biology, Ghent, Belgium; [3]CSIRO Agriculture, St Lucia, Queensland, Australia

18:00 **36** **Elucidating the mechanisms of *Beauveria bassiana* induced plant resistance -** Maya Raad, Travis Glare, Michael Rostás; Bio-Protection Research Centre, Lincoln University, Lincoln, New Zealand

CONTRIBUTED PAPERS 16:30-18:30 - *Courteline*

Diseases of Beneficial Invertebrates 1

David Bass

16:30 **37-STU** **Flat oyster follows the apoptosis pathway to defend against the protozoan parasite *Bonamia ostreae* -** Ophélie Gervais[1], Chollet Bruno[1], Tristan Renault[2], Isabelle Arzul[1]; [1]Laboratoire de Génétique et Pathologie des Mollusques Marins, IFREMER, La Tremblade, France; [2]Département Ressources Biologiques et Environnement, IFREMER, Nantes, France

16:45 **38** **Influence of temperature on the haplosporidian parasite *Bonamia ostreae* exposed to *Crassostrea gigas* and *Ostrea edulis* oyster mucus** - Sergio Fernandez-Boo, Ophélie Gervais, Bruno Chollet, Isabelle Arzul; Laboratoire de Génétique et Pathologie des Mollusques Marins, IFREMER, La Tremblade, France

17:00 **39** **Monitoring of the autophagy pathway in *C. gigas* during an experimental OsHV-1 infection at cellular molecular and proteomic levels** - Sandy Picot[1], Benjamin Morga[1], Nicole Faury[1], Isabelle Arzul[1], Tristan Renault[2]; [1]Laboratoire de Génétique et Pathologie des Mollusques Marins, IFREMER, La Tremblade, France; [2]Département Ressources Biologiques et Environnement, IFREMER, Nantes, France

17:15 **40** **Genome sequencing of the Ostreid herpesvirus 1 infecting oysters in Tomales Bay, California** - Colleen Burge, Stanley Langevin, Collin Closek, Natalie Rivlin, Carolyn Friedman; Institute of Marine and Environmental Technology, University of Marine Baltimore County, United States

17:30 **41 Reducing the impact of pathogens and disease in the Irish Pacific oyster *Crassostrea gigas* by understanding Environment: Host/Pathogen interaction** - Babette Bookelaar, Sharon Lynch, Sarah Culloty; University College Cork, Ireland

17:45 **42 Apicomplexans infecting marine molluscs** - Mark Freeman[1], Arni Kristmundsson[2]. [1]Ross University School of Veterinary Medicine, Saint Kitts and Nevis; [2]Institute for Experimental Pathology at Keldur, University of Iceland, Iceland

18:00 **43 Is an apicomplexan responsible for the collapse in the Iceland scallop stock in Iceland?** - Arni Kristmundsson[1], Asthildur Erlingsdottir[1], Mark Freeman[2]; [1]Institute for Experimental Pathology at Keldur, University of Iceland, Reykjavík, Iceland; [2]Ross University School of Veterinary Medicine, Saint Kitts and Nevis

CONTRIBUTED PAPERS 16:30-18:30 - *Vouvray*

Virus 2

Robert Harrison & Bergmann Ribeiro

16:30 **44-STU Genome stability of AgseNPV-B after serial in vitro passage** - Gianpiero Gueli Alletti, Eric Carstens, Johannes Jehle; Julius Kühn-Institut, Federal Research Centre for Cultivated Plants, Institute for Biological Control, Darmstadt, Germany

16:45 **45-STU Synthetic baculovirus genomes to extend host range** - Yu Shang[1], Fei Deng[1], Geng Xiao[1], Man Wang[1], Dian Hou[1], Kai Pan1, Basil Arif[2], Hua Wang[1], Zhi Hu[1]; [1]State Key Laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, China; [2]Great Lakes Forestry Centre, Sault Ste. Marie, Canada

17:00 **46-STU Genotype Detection and Abundance within Baculoviruses using Next Generation Sequencing** - Christopher Noune, Caroline Hauxwell; Queensland University of Technolgy, Brisbane, Australia

17:15 **47 The complete genome sequence of Plodia interpunctella granulovirus: discovery of an unusual inhibitor of apoptosis (IAP) gene** - Robert L. Harrison[1], Daniel L. Rowley[1], C. Joel Funk[2]; [1]Invasive Insect Biocontrol and Behavior Laboratory, USDA-ARS Beltsville, Maryland, United States; [2]Department of Biology, John Brown University, Siloam Springs, Arkansas, United States

17:30 **48-STU Study of the domestication of a viral genome in the parasitoid wasp *Venturia canescens*** - Matthieu Leobold[1], Annie Bézier[1], Anne-Nathalie Volkoff[2], Jean-Michel Drezen[1]; [1]Institut de recherche sur la biologie de l'insecte, CNRS UMR7261, Université François Rabelais, Tours, France; [2]Diversité, Génomes Interactions Microorganismes - Insectes, INRA UMR1333, Université Montpellier 2, France

17:45 **49-STU Adaptation genomics in the bracovirus of *Cotesia sesamiae* -** Jeremy Gauthier[1], Philippe Gayral[1], Bruno Le Ru[2], Stephane Dupas[3], Severine Jancek[1], Gabor Gyapay[4], Laure Kaiser[3], Elisabeth Herniou[1]; [1]Institut de recherche sur la biologie de l'insecte - CNRS UMR7261, Université François Rabelais, Tours, France; [2]International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya; [3]Evolution Génomes Comportement Ecologie CNRS UMR9191 Gif-sur-Yvette, France; [4]Centre National de Séquençage Institut de génomique Direction des Sciences du vivant CEA, Evry, France

18:00 **50 Expansion of the family *Nimaviridae*** - Kelly S. Bateman[1], Ronny Van Aerle[1], Rose Kerr[1], Jamie Bojko[1], K. Fraser Clark[2,3], Sarah E. Stewart-Clark[3], Philip Byrne[4], Spencer J. Greenwood[2], David Bass[1], Grant D. Stentiford[1]; [1]European Union Reference Laboratory for Crustacean Diseases, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Weymouth, United Kingdom; [2]Dept. of Biomedical Sciences and AVC Lobster Science Centre, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Canada; [3]Department of Plant and Animal Sciences, Agricultural Campus, Dalhousie University, Truro, Canada; [4]Department of Fisheries and Oceans Canada, Charlottetown, Canada

18:00 **51 Characterization and complete genome sequence of a new cypovirus isolated from *Thyrinteina arnobi* (Stoll, 1782) (Lepidoptera: *Geometridae*)** - André Horta[1], Daniel Ardisson-Araújo[2], Fabricio Morgado[2], Leonardo Silva[2], Fernando Melo[2], Manoel Victor Lemos[3], Zulene Ribeiro[3], Arlindo Junior[3], Carlos Wilcken, Bergmann Ribeiro[2]; [1]Universidade Estadual Paulista "Júlio de Mesquita Filho", Faculdade de Ciências Agronômicas Botucatu, Brazil; [2]Universidade de Brasília, Instituto de Biologia, Departamento de Biologia Celular, Brasilia, Brazil; [3]Universidade Estadual Paulista "Júlio de Mesquita Filho", Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal, Brazil

CONTRIBUTED PAPERS 16:30-18:30 - *Chinon*

Microbial Control 1

Travis Glare

16:30 **52 Investigations on spore residues of the product XenTari® (*Bacillus thuringiensis subsp. aizawai*) and their persistence on sweet pepper and tomato** - Dietrich Stephan, Alexandra Wagner; Institute for Biological Control, Julius Kühn-Institut (JKI) Darmstadt, Germany

16:45 **53 Encapsulation and UV Photoprotection of a Vip3 toxin** - Inigo Ruiz De Escudero[1,2], Leopoldo Palma[3], Francisco Mañeru[1], Primitivo Caballero[1]; [1]Instituto de Agrobiotecnología, CSIC-UPNA, Gobierno de Navarra, Spain; [2]Universidad Pública de Navarra. Laboratorio de Entomología Agrícola y Patología de Insectos, Pamplona, Spain; [3]Facultad de Ciencias Agrarias, Universidad Nacional del Litoral, Santa Fe, Argentina

17:00 **54-STU Genetic and biological characterization of *Bacillus thuringiensis* isolates showing insecticidal activity against *Leptinotarsa decemlineata* (Coleoptera: *Chrysomelidae*)** - Mikel Dominguez[1,2], Jesus Murillo[1], Primitivo Caballero[1,2]; [1]Departamento de Produccion Agraria, Universidad Publica de Navarra (UPNA) Pamplona, Navarra, Spain; [2]Instituto de Agrobiotecnologia CSIC-Gobierno de Navarra, Navarra, Spain

17:15 **55-STU Analysis of the occurrence of cry, vip3 and chitinases genes in *Bacillus thuringiensis* strains isolated from Algeria -** Zahia Djenane[1,2,3], Joaquín Gomis-Cebolla[2], Fairouz Elaichar[1], Hassiba Khorf Khorf[1], Ahmed Abderrahmani[1], Farida Nateche[1], Juan Ferré[2]; [1]Laboratory of Cellular and Molecular Biology, team of microbiology, Algiers, Algeria; [2]ERI de Biotecnología y Biomedicina, Universitat de València, Burjassot, Spain; 3 University of Science and Technology, Algiers, Algeria

17:30 **56-STU Increase toxicity from a modified Cry3Aa toxin against *Monochamus alternatus;*** Yajie Guo[1], Yafang Wang[2], Zhuoying Xu2, Yueting Xiong[2], Yani Mou[2], Qiannan Lin[1], Rong Wang1, Xia Hu[1], Guanghong Liang[1], Xiong Guan[2], Songqing Wu[1,2], Feiping Zhang[1]; [1]Collage of Forestry, Fujian Agriculture and Forestry University, Fuzhou, People's Republic of China; [2]Key Laboratory of Biopesticide and Chemical Biology, Ministry of Education, Fujian Agriculture and Forestry University, Fuzhou, People's Republic of China

17:45 **57-STU Spanish strains of *Bacillus thuringiensis* as biological control agents against *Lobesia botrana* (Lepidoptera: Tortricidae) larvae** - Javier Caballero[1,2], Isabel Matas[1], Maite Zarranz[1], Primitivo Caballero[1,2]; [1]Instituto de Agrobiotecnologia CSIC-Gobierno de Navarra (IDAB) Mutilva, Navarra, Spain; [2]Departamento de Produccion Agraria, Universidad Publica de Navarra (UPNA) Pamplona, Navarra, Spain

18:00 **58 New Insecticidal Proteins from Non-*Bacillus thuringiensis* Microbial Diversity - -**Lu Liu[1], Jarred Oral[1], Dan Altier[2], Jessica O'rear[1], Barbara Rosen[1], James Le[1], Mark Mcdonald[1], David Cerf[1], Jon Robeson[2], Lisa Procyk[2], Adane Kassa[2], Weiping Xie[1], Genhai Zhu[1], Jennifer Barry[2], Claudia Pérez-Ortega[2], Nuria Jiménez-Juárez[2], Miles Cowart[2], Jian-Zhou Zhao[2], Ute Schellenberger[1], Nasser Yalpani[2], Jun-Zhi Wei[1], Virginia Crane[2], Gary Sandahl[2], Mark Nelson[2], Albert Lu[2], Gusui Wu[2], [1]DuPont Pioneer, Hayward, CA, United States; [2]DuPont Pioneer, Johnston, United States

18:15 **59 Managing the evolution of resistance to biopesticides with genetically modified insects** - Raymond Ben; University of Exeter, Penryn campus Cornwall, United Kingdom

18:30-19:45

Division Business Meetings and Workshops

Bacteria Division Meeting *Vouvray*

Fungi Division Meeting *Chinon*

Microsporidia Division Meeting *Courteline*

'Open discussion on hot topics in microsporidia research'
Y. Sokolova

TUESDAY – 26 July

07:00-08:00 **Qi Gong** *Foyer*
07:30-12:30 **Registration** *Hall*

SPECIAL SYMPOSIUM 08:00-10:00 - *Vouvray*

Human impact on pathogens-honeybee interactions

Aurore Dubuffet & Philippe Gayral

08:00 **60 EPILOBEE: Results from a pan-European epidemiological study on honeybee colony losses 2012-2014, conducted by the European Union Reference Laboratory -** Marie-Pierre Chauzat, Marion Laurent, Antoine Jacques, Epilobee Consortium, Laura Cauquil, Marie-Pierre Riviere, Mathilde Saussac, Stéphanie Bougeard, Pascal Hendrikx, Magali Chabert, Honey Bee Patholohy Unit, European Union Reference Laboratory for Honey Bee Health (Anses) French Agency for Food, Environmental and Occupational Health Safety, Sophia Antipolis, France

08:30 **61 Honey bee stressor interactions: never the same, and so much to learn** - Geoffrey Williams; University of Bern and Agroscope, Switzerland

62 Cancelled

63 Cancelled

09:00 **124-STU *In-vitro* transmission of the Chronic Bee Paralysis Virus and co-exposition with a neonicotinoid in the honeybee -** Marianne Coulon[1,2], Frank Schurr[1], Nicolas Cougoule[1], Anne Dalmon[2], Cédric Alaux2, Yves Le Conte[2], Richard Thiery[1], Magali Ribiere-Chabert[1], Eric Dubois[1]; [1]Laboratoire de Sophia Antipolis, Anses, Sophia-Antipolis, France; [2]INRA PACA, Abeilles et environnement, UR406 Avignon, France

09:15 **64 Man-made epidemics: Varroa and DWV in honeybees and the risk they pose to wild pollinators** - Lena Wilfert[1], Robyn Manley[1], Mike Boots[2]; [1]Centre for Ecology and Conservation, University of Exeter, Penryn Campus, United Kingdom; [2]Integrative Biology, UC Berkeley, United States

CROSS DIVISION SYMPOSIUM 08:00-10:00 - *Chinon*

Recruitment of beneficial microbes and nematodes

Ivan Hiltpold, Mike Brownbridge & David Shapiro

08:00 **65 Entomopathogenic fungi: Friend or enemy of the plant?** - Rob Van Tol[1], Gerrie Wiegers[1], Marilena Palmisano[2], Jurg Grunder[2]; [1]Wageningen University and Research Centre, Wageningen, Netherlands; [2]Zurich University of Applied Sciences Waedenswil, Switzerland

08:30 **66 Potential of root-associated pseudomonads with insecticidal activity for biological control of soil-dwelling insect pests of crops -** Christoph Keel[1], Nicola Imperiali[1], Geoffrey Jaffuel[2], Pascale Flury[3], Monika Maurhofer[3], Ted Turlings[2]; [1]University of Lausanne, Department of Fundamental Microbiology, Lausanne, Switzerland; [2]University of Neuchatel, Fundamental and Applied Research in Chemical Ecology, Neuchatel, Switzerland; [3]Swiss Federal Institute of Technology Zürich, Switzerland

09:00 **67 Threesome in the rhizosphere: bacteria, entomopathogenic nematode, and plant interactions** - Ivan Hiltpold[1], Michael Brownbridge[2]; [1]Western Sydney University, Australia; [2]Vineland Research and Innovation Centre, Canada

09:30 **68 Entomopathogenic Nematodes Boost Plant Immunity -** Parwinder Grewal; University of Texas Rio Grande Valley, Edinburg, United States

CONTRIBUTED PAPERS 08:00-10:00 - *Courteline*

Bacteria 2

Marianne Carey & Shuyuan Guo

08:00 **69 Cry1Ac toxin mode of action in heliothines** - Heba Abdelgaffar[1], Cris Oppert[2], Jessica Monserrate[2], Juan Luis Jurat-Fuentes[1]; [1]Dept of Entomology and Plant Pathology, University of Tennessee, Knoxville, United States; [2]Bayer CropScience, Morrisville NC, United States

08:15 **70 In-plant protection from the insect pest *Helicoverpa armigera* by trans-kingdom RNAi -** Julia Bally[1], Glen Mcintyre[2], Rachel Doran[1], Ignacio Larrinua[3], Kenneth Narva[3], Peter Waterhouse[1,2]; [1]Centre for Tropical Crops and Biocommodities, Brisbane, Australia; [2]University of Sydney, Australia; [3]Dow AgroSciences, United States

08:30 **71 *Lysinibacillus sphaericus* Binary toxin structure revealed in situ by *de novo* phasing with an X-ray free-electron laser: Insights into the larvicidal biology of BinA and BinB -** Jacques-Philippe Colletier[1], Michael Sawaya[2], Jose Rodriguez[2], Duilio Cascio[2], Dennis Bideshi[3], Robert Hice[3], Brian Federici[4], David Eisenberg[2]; [1] Institut de Biologie Structurale, Université Joseph Fourier, Grenoble I, CNRS UMR5075, CEA, France; [2]UCLA-DOE Institute of Genomics and Proteomics, University of California, Los Angeles, United States; [3]Department of Entomology, University of California, Riverside, United States; [4]Department of Entomology, Institute of Integrative Genome Biology, University of California, Riverside, United States

08:45 **72 A binB knockout in *Lysinibacillus sphaericus* demonstrates BinA can form a crystal without BinB *in Bacillus thuringiensis* -** Hyun-Woo Park[1], Dennis Bideshi[1], Brian Federici[2]; [1]Department of Entomology, University of California, Riverside, and Department of Biological Sciences, California Baptist University, Riverside, California, United States; [2]Interdepartmental Graduate Programs in Microbiology and Cell, Molecular, and Developmental Biology, University of California, Riverside, United States

09:00 **73 Structure and activity of the Cry6Aa pesticidal toxin** - Colin Berry, Alexey Dementiev, Jason Board, Anand Sitaram, Timothy Hey, Matthew Kelker, Xiaoping Xu, Yan Hu, Cristian Vidal-Quist, Vimbai Chikwana, Samantha Griffin, David Mccaskill, Nick Wang, Shao-Ching Hung, Michael Chan, Marianne Lee, Jessica Hughes, Alice Wegener, Raffi Aroian, Kenneth Narva; School of Biosciences, Cardiff, United Kingdom

09:15 **74 The *Bacillus thuringiensis* toxin Cry6Aa1 forms ionic channels in giant liposomes** - Vincent Vachon[1], Maxime Schmidt[1], Timothy Hey[2], Xiaoping Xu[2], Samantha Griffin[2], Vimbai Chikwana[2], David McCaskill[2], Ken Narva[2], Jean-Louis Schwartz[1,3]; [1]Groupe d'étude des protéines membranaires, Département de physiologie moléculaire et intégrative, Université de Montréal, Canada; [2]Dow Agrosciences LLC, Indianapolis, United States ; [3]Centre SEVE de recherche en sciences du végétal, Université de Sherbrooke, Canada

09:30 **75-STU Two polysaccharides are involved in the formation of specific biofilm structures in *B. thuringiensis* -** Racha Majed[1,2], Mireille Kallassy[2], Michel Gohar[1]; [1]MICrobiologie de l'ALImentation au Service de la Santé humaine, AgroParisTech, INRA UMR1319, Jouy-en-Josas, France; [2]Laboratoire de Biotechnologie, Université Saint-Joseph, Beyrouth, Lebanon

09:45 **76 Enzymatic activity of *Bacillus thuringiensis* toxins** - David Pauron, Marcel Amichot, Marie-Paule Esposito, Armel Gallet; Institut Sophia Agrobiotech, Equipe Bioinsecticides, Environnement et Santé, CNRS UMR7254, Université de Nice Sophia-Antipolis, INRA UMR1355, Sophia Antipolis, France

CONTRIBUTED PAPERS 08:00-10:00 - *Bourgueil*

Fungi 1

Ann Hajek & Helen Hesketh

08:00 **77 *Entomophaga maimaiga* in *Lymantria dispar* in Eastern Europe** - Ann Hajek[1], Daniela Pilarska[2,3], Milan Zubrik[4]; [1]Department of Entomology, Cornell University, Ithaca, New York, United States; [2]New Bulgarian University, Department of Natural Sciences Sofia, Bulgaria; [3]Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences Sofia, Bulgaria; [4]National Forest Centre, Forest Research Institute, Forest Protection and Game Management, Banska Stiavnica, Slovakia

08:15 **78 Termite choice of direction to pathogen odor related with nestmate olfactory signals -** Aya Yanagawa[1], Tomoya Imai[1], Toshiharu Akino[2], Toshimitsu Hata[1], Tsuyoshi Yoshimura[1], Fumio Yokohari[3], Yoshihiro Toh[4]; [1]Kyoto University Japan; [2]Kyoto Institute of Technology, Japan; [3]Fukuoka University, Japan; [4]Kyushu University, Japan

08:15 **79-STU Natural occurrence of *Beauveria bassiana* in soil, as infections in stink bugs and as endophytes in bean plants, from organic and conventional fields in Cuba** - Yordanys Ramos[1], Orelvis Portal[2], Erik Lysøe[3], Annarella Chea[1], Luis Rojas[4], Nicolai Meyling[5], Ingeborg Klingen[3]; [1]Department of Agronomy, Universidad Central "Marta Abreu" de Las Villas (UCLV), Santa Clara, Cuba; [2]Department of Biology, UCLV, Santa Clara, Cuba; [3]Norwegian Institute of Bioeconomy, Biotechnology and Plant Health Division, As, Norway; [4]Instituto de Biotecnologia de las Plantas, UCLV, Santa Clara, Cuba; [5]Department of Plant and Environmental Sciences, University of Copenhagen, Frederiksberg, Denmark

08:45 **80-STU** **Biotic and abiotic factors influencing the virulence of Entomophthoromycota on aphids in cereals** - Stéphanie Saussure[1], Cecilie Sletteng[2], Nina Trandem[1,2], Karin Westrum[1], Ingeborg Klingen[1]; [1]Norwegian Inst. of Bioeconomy Research, Biotechnology and Plant Health Division, Ås, Norway; [2]Norwegian Univ. of Life Sciences, Ås, Norway

09:00 **81** **Entomophthoromycota pathogens of insects from Argentina: an updated review** - Claudia Lopez Lastra, Romina Manfrino, Alejandra Gutierrez; Centro de estudios parasitologicos y de vectores, Argentina

09:15 **82-STU** **Incidence of fungal infections on Neotropical ants: environment or the host, which has more influence? -** José Pablo Barrantes[1], Maria José Monge-Salazar[1], Milagro Granados-Montero[2], Priscila Chaverri[1,3]; [1]Escuela de Biología, Universidad de Costa Rica, San José, Costa Rica; [2]Centro de Investigaciones en Protección de Cultivos, Escuela de Agronomía, Universidad de Costa Rica, San José, Costa Rica; [3]Department of Plant Science and Landscape Architecture, University of Maryland, Maryland, United States

09:30 **83-STU** **Impacts of conventional and organic agriculture on soil-borne entomopathogenic fungi -** Eric Clifton[1], Stefan Jaronski[2], Erin Hodgson[1], Aaron Gassmann[1]; [1]Iowa State University, United States; [2]United States Department of Agriculture, Agricultural Research Service, United States

09:45 **84** **Effect of a predatory mite on transmission of the fungus *Neozygites floridana* in *Tetranychus urticae* populations -** Nina Trandem[1], Ronny Berdinesen[1], Judith Pel[!2], Ingeborg Klingen[3]; [1]Norwegian University of Life Sciences, Norway; [2]J.K. Pell Consulting, United Kingdom; [3]Norwegian Institute of Bieconomy Research, Norway

10:00-10:30 **COFFEE BREAK** *Agnès Sorel*

MICROBIAL CONTROL DIVISION 10:30-12:30 - *Chinon*
SYMPOSIUM

Next Generation Biopesticides

Carrie Hauxwell

10:35 **85** **Using bumblebees for targeted application of biopesticides**
Sarah Van Beneden, Soraya Franca Marlies Vleugels, Felix Wäcker; Biobest Ilse Velden Westerlo, Belgium

11:00 **86** **Working with insect pathogen ecology for better biocontrol delivery -** Michael Brownbridge[1], Travis Glare[2]**;** [1]Vineland Research and Innovation Centre, Vineland Station, Canada; [2]Bio-Protection Research Centre, Lincoln University, Lincoln, New Zealand

11:25 **87** **Characteristics of novel bacterial insecticides/miticides/ nematicides from *Chromobacterium subtsugae* and *Burkholderia rinojensis* -** Timothy Johnson, Pamela Marrone; Marrone Bio Innovations, United States

11:50 **88** **Regulatory implications of new technologies -** Roma Gwynn; Rationale, United Kingdom

CONTRIBUTED PAPERS 10:30-12:30 - *Vouvray*

Virus 3

Robert Possee & Jenny Cory

10:30 **89-STU** **RACK1, a ribosomal protein involved in signaling, stress response and viral translation** - Evelyne Einhorn, Franck Martin, Carine Meignin, Jean-Luc Imler; Institut de biologie moléculaire et cellulaire, CNRS UPR9022, Strasbourg, France

10:45 **90-STU** **Resistance to baculoviruses in the midgut of *Adoxophyes honmai*** - Kento Iwata, Yasuhisa Kunimi, Maki N. Inoue, Madoka Nakai; Tokyo University of Agriculture and Technology, Japan

11:00 **91-STU** **Insect immune system to determine baculoviruses host specificity -** Yu-Wei Chen, Yueh-Lung Wu; Department of Entomology, National Taiwan University, Taiwan

11:15 **92-STU** **The role of pathogen diversity on the evolution of resistance in an insect -** Leon Yu Zheng Li, Jenny Cory; Simon Fraser University (SFU) – Department of Biological Sciences, Burnaby, British Columbia, Canada

11:30 **93** **Pathogen competition in the cabbage looper, *Trichoplusia ni*: are multiple pathogens more effective than one? -** Jennifer Scholefield, Jenny Cory; Simon Fraser University (SFU) – Department of Biological Sciences, Burnaby, British Columbia, Canada

11:45 **94** **Successive passages of mixed genotype virus populations: influence of the insect host colony -** Benoit Graillot[1], Christine Blachere-Lopez[1,3], Samantha Besse[2], Myriam Siegwart[4], Miguel Lopez-Ferber[1]; [1]Laboratoire de Génie de l'Environnement Industriel, Ecole Nationale Supérieure des Mines d'Alès, France; [2]Natural Plant Protection, Arysta LifeSciences, Pau, France; [3]INRA, Alès, France; [4]Plant Pathology Unit UR 407 INRA Montfavet, France

12:00 **95** **Observations on a persistent baculovirus infection of *Trichoplusia ni* cells in culture -** Robert Possee, Sarah Irons, Linda King; Oxford Brookes University, Headington Campus, Oxford, United Kingdom

12:15 **96** **Molecular response of *Manduca sexta* immune tissues to parasitization by the bracovirus associated wasp *Cotesia congregata* -** Germain Chevignon[1], Sébastien Cambier[2], Corinne Da Silva[3], Julie Poulain[3], Sébastien Moreau[1], Jean-Michel Drezen[1], Elisabeth Huguet[1]; [1]Institut de recherche sur la biologie de l'insecte, CNRS UMR7261, Université François Rabelais, Tours, France; [2]Luxembourg Institute of Science and Technology, Belvaux, Luxembourg; [3]Genoscope-Centre national de séquençage, CEA Evry, France

CONTRIBUTED PAPERS 10:30-12:30 - *Bourgueil*

Microsporidia

Susan Bjornson

10:30 **97** **Microsporidia – Emergent Pathogens in the Global Food Chain -** Grant Stentiford; Centre for Environment, Fisheries and Aquaculture Science, Weymouth, Dorset, United Kingdom

10:45 **98-STU** **Genome evolution and pre-mRNA splicing in microsporidia and early-diverging fungi -** Thomas A. Whelan[1], Nicole T. Lee[1], C. Alisha Quandt[2], Timothy Y. James[2], Naomi M. Fast1; [1]Biodiversity Research Centre and Department of Botany, University of British Columbia, Canada; [2]Department of Ecology and Evolutionary Biology, University of Michigan, United States

11:00 **99** **Molecular phylogenetics as a reason for redefinition of two classical genera of Microsporidia: *Nosema* and *Vairimorpha* -** Yuri Tokarev[1], Charles Vossbrinck[2]; [1]All-Russian Institute of Plant Protection, Russia; [2]The Connecticut Agricultural Experiment Station, United States

11:15 **100** **Transcriptome and Prokaryotic Expression Analysis of HMG1 of *Nosema bombycis* -** Jiping Liu[1,2]; [1]College of Animal Science South China Agricultural University, Wushan, Guangzhou, China; [2]Regional Sericulture Training centre for Asia and Pacific, South China Agriculture University, Wushan, Guangzhou, China

11:30 **101** **Specific nested-PCR and LAMP methods to detect the spore wall protein gene of *Enterocytozoon hepatopenaei* that causes slow growth in penaid shrimp -** Pattana Jaroenlak[1,2], Piyachat Sanguanrut[2,3], Paul Salachan[2], Bryony Williams[4], Grant Stentiford[5], Timothy Flegel[6], Kallaya Sritunyalucksana[3,6], Ornchuma Itsathitphaisarn[1,2]; [1]Department of Biochemistry, Faculty of Science, Mahidol University, Bangkok, Thailand; [2]Center of Excellence for Shrimp Molecular biology and Biotechnology, Faculty of Science, Mahidol University, Bangkok, Thailand; [3]Shrimp–Virus Interaction Laboratory, National Center for Genetic Engineering and Biotechnology, Bangkok, Thailand; [4]Biosciences, College of Life and Environmental Sciences, University of Exeter, Devon, United Kingdom; [5]CEFAS, Weymouth, Dorset, United Kingdom; [6]National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, Thailand Science Park, Pathumthani, Thailand

11:45 **102** **A pesticide and an intracellular microsporidial parasite target *Drosophila* lipid stores in an unusual "competition" between a natural pathogen and an environmental poison -** Adrien Franchet[1], Sebastian Niehus[1], Dominique Ferrandon[1,2]; [1]University of Strasbourg Institute for Advanced Study (USIAS), CNRS UPR9022, France; [2]Institut de Biologie Moléculaire et Cellulaire (IBMC) – CNRS - Strasbourg, France

12:00 **103** ***Hyperspora aquatica* n.gn., n.sp., a hyperparasitic microsporidian infecting paramyxid protists is closely related to crustacean-infecting taxa -** Grant Stentiford; CEFAS, Weymouth, Dorset, United Kingdom

CONTRIBUTED PAPERS 10:30-12:30 - *Courteline*

Bacteria 3

Baltasar Escriche & Ming Sun

10:30 **104** **Resistance to Bt maize by western corn rootworm: inheritance, fitness costs and cross-resistance** - Aaron

Gassmann; Iowa State University, United States

10:45 **105** **Insect resistance to *Bacillus thuringiensis* Cry3Aa toxin is associated with a novel ABC protein -** Yannick Pauchet[1], Anne Bretschneider[1], Sylvie Augustin[2], David Pauron[3], David Heckel[1]; [1]Max Planck Institute for Chemical Ecology, Jena, Germany; [2]INRA Orléans UR0633, France; [3]Institut Sophia Agrobiotech, INRA PACA, Sophia Antipolis, France

11:00 **106-STU** **The limited role of *Bombyx mori* ABCC3 as a Cry toxin receptor in comparison to ABCC2 -** Haruka Endo[1,2], Fumika Ichino[1], Satomi Adegawa[1], Hiroko Tabunoki1, Ryoichi Sato[1]; [1]Tokyo University of Agriculture and Technology, Japan; [2]Research Fellow of Japan Society for the Promotion of Science, Japan

11:15 **107-STU** **Multi-binding ability to functional receptors and BmABCC2 dependent cytotoxicity-relevant property of the domain II loop region of Cry1Aa -** Satomi Adegawa, Shingo Kikuta, Ryoichi Sato; Graduate School of Bio-Applications and Systems Engineering, Tokyo University of Agriculture and Technology, Japan

11:30 **108** **Comparative Analysis of Gene Expression Profiles in Cry1Ac Resistant and Susceptible Strains of *Heliothis virescens***
Omaththage Perera†, Cris Oppert, Jerreme Jackson, Anaïs Castagnola, Juan Luis Jurat-Fuentes; United States Department of Agriculture, Agricultural Research Service, Southern Insect Management Research Unit Stoneville, MS, United States

12:00 **109** **Differential induction of immune system related genes in *Spodoptera exigua* after Vip3Ca challenge -** Patricia Hernandez-Martinez, Baltasar Escriche; Departmento de Genética, and Estructura de Recerca Interdisciplinar en Biotecnologia i Biomedicina, Universitat de València, Spain

12:00 **110** **Microevolutionary mechanisms of wax moth *Galleria mellonella* resistance to *Bacillus thuringiensis* -** Ekaterina Grizanova[1], Tariq Butt[2], Andreas Vilcinskas[3], Ivan Dubovskiy[1]; [1]Institute of Systematic and Ecology of Animals, Siberian Branch of Russian Academy of Sciences, Novosibirsk, Russia; [2]Dept of Biosciences, College of Science, Swansea University, United Kingdom; [3]Institute for Insect Biotechnology, Justus-Liebig University, Giessen, Germany

12:15 **111** **Fitness costs associated with multi-toxin resistance in the cabbage looper (*Trichoplusia ni*) -** Guillaume Tetreau[1,2], Ran Wang[1,3,4], Ping Wang[1]; [1]Department of Entomology, Cornell University, New York State Agricultural Experiment Station, NY, United States; [2]CNRS-IFREMER: UMR5244, Université de Perpignan Université de Montpellier, OMS/WHO, Perpignan, France; [3]Department of Entomology, Nanjing Agricultural University, Nanjing, China; [4]Institute of Plant and Environment Protection, Beijing Academy of Agriculture and Forestry Sciences, Beijing, China

OPTIONAL EXCURSION TO AMBOISE

12:30-13:00	Distribution Lunch bag	*Hall*
13:00-17:30	Excursion to Amboise Castle and le Clos Lucé	

GARDEN PARTY AT THE DOMAINE DE CANDÉ

16:30	Bus departure	*Outside Vinci*
17:30 & 18:30	Optional castle visit	
18:00	5K Race	
19:00	Barbecue	

WEDNESDAY – 27 July

07:30-08:30	**Qi Gong**	*Foyer*
08:00-17:00	**Registration**	*Hall*

CROSS DIVISION SYMPOSIUM 08:30-10:30 - *Chinon*

Next Generation Sequencing

David Bass & Helen Hesketh

08:30 **112** **High-Throughput Sequencing and Bioinformatic Tools for Invertebrate Pathology - an Overview -** Ronny Van Aerle; European Union Reference Laboratory for Crustacean Diseases, Centre for Environment, Fisheries and Aquaculture Science, Weymouth, United Kingdom

09:00 **113** **Next generation sequencing - a powerful approach to assess potential effects of BCAs on microbial communities in soil -** Juerg Enkerli[1], Johanna Mayerhofer[1], Franco Widmer[1], Martin Hartmann[2]; [1]Institute for Sustainability Sciences Agroscope, Zürich, Switzerland; [2]Forest Soils and Biogeochemistry, Swiss Federal Research Institute WSL, Birmensdorf, Switzerland

09:30 **114** **Using dual-RNAseq to study host-pathogen interactions: data generation and analysis -** Henrik Hjarvard De Fine Licht; Section for Organismal Biology, Department of Plant and Environmental Sciences, University of Copenhagen (PLEN), Frederiksberg, Denmark

10:00 **115** **Big data and little *Metarhizium*: evolution and interactions of an endophytic insect pathogenic fungus -** Brian Lovett, Raymond St. Leger; Department of Entomology, University of Maryland (UMD), College Park, MD, United States

CONTRIBUTED PAPERS 08:45-10:30 - *Bourgueil*

Nematodes

David Shapiro-Ilan

09:00 **116** **Dissecting the immune defence against the entomopathogenic nematodes: fluctuation of Cecropin and haemocytes during infection of *Spodotera exigua* -** Reyhaneh Darsouei, Javad Karimi; Ferdowsi University of Mashhad (FUM), Biocontrol and Insect Pathology Lab, Dept of Plant Protection, School of Agriculture, Mashhad, Iran

09:15 **117** **Who one associates with matters: Role of *Xenorhabdus bovienii* (Enterobacteriaceae) symbionts on the fitness of its *Steinernema* nematode hosts** - S.Patricia Stock, John Mcmullen; Department of Entomology, University of Arizona Tucson, AZ, United States

09:30 **118** **Curative Control of the Peachtree Borer Using Entomopathogenic Nematodes -** David Shapiro-Ilan[1], Ted Cottrell[1], Russ Mizell III[2], Dan Horton[3]; [1]USDA-ARS, United States; [2]University of Florida, United States; [3]University of Georgia, United States

09:45 **119-STU** **Attraction of entomopathogenic nematode isolates to insect feeding is driven by their previous association with wild or domesticated highbush blueberry (*Vaccinium corymbosum*) plants -** Monique Rivera, Albrecht Koppenhofer; Rutgers University, United States

120-STU Cancelled

121-STU **moved to Fungi 2**

10:00 **122** **Potential of entomopathogenic fungi and nematodes against *Parahypopta caestrum* in laboratory assays -** Monica Oreste[1], Eustachio Tarasco[1], Luca Ruiu[2]; [1]Department of Soil, Plant and Food Science, University of Bari Aldo Moro (DISSPA) – Italy; [2]Department of Agriculture, University of Sassari – Italy

10:15 **123** **Microbial control of *Cossus cossus* with entomopathogenic nematodes and fungi in laboratory and field assays -** Rocco Addante[1], Monica Oreste[1], Angela D'Accolti[1], Luca Ruiu[2], Eustachio Tarasco[1]; [1]Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, Italy; [2]Department of Agriculture, University of Sassari, Italy

CONTRIBUTED PAPERS 08:45-10:30 - *Courteline*

Virus 4

Bryony Bonning & Anne Dalmon

124-STU moved to Special Symposium

08:45 **125-STU** **Diversity and evolution of Sinaivirus and related viruses in honeybees and wild hymenoptera -** Diane Bigot1, Elisabeth Herniou[1], Anne Dalmon2, Nicolas Galtier[3], Philippe Gayral[1]; [1]Institut de recherche sur la biologie de l'insecte, CNRS UMR7261, Université François Rabelais, Tours, France; [2]INRA PACA, Abeilles et environnement, UR406 Avignon, France; [3]ISEM, Université Montpellier II, CNRS UMR5554, Montpellier, France

09:00 **126-STU** **An opposite effect of *Dicistroviridae* on the RNA interference defense mechanism of their host, *Bombus terrestris* -** Kaat Cappelle[1], Guy Smagghe[1], Maarten Dhaenens[2], Ivan Meeus[1]; [1]Department of Crop Protection, Faculty of Bioscience Engineering, Universiteit Gent, Belgium; [2]Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Universiteit Gent, Belgium

09:15 **127** **Honeybee (*Apis mellifera*) viruses or bee (Apiformes) viruses? -** Anne Dalmon, Virginie Diévart, Maxime Thomasson, Bernard Vaissière, Yves Le Conte, Laurent Guilbaud, Mickaël Henry; INRA PACA, Abeilles et environnement, UR406 Avignon, France

09:30 **128** **Infection dynamics of honeybee viruses in AmE-711 cells** - Jimena Carrillo-Tripp[1], Michael Goblirsch[2], Adam Dolezal[1], W. Miller[1], Amy Toth[1], Bryony Bonning[1]; [1]Iowa State University, United States; [2]University of Minnesota, United States

09:45 **129** **Occurrence, Pathology, and Ultrastructure of an Iridovirus and Cytoplasmic Polyhedrosis Virus Occurring in Daphnids in the Czech Republic** - Jirı Vavra[1], Bily Tomas[1], Jana Nebesrova[1], Brian Federici[2]; [1]Faculty of Science, Charles University, Praha, Czech Republic; [2]University of California, Riverside, United States

10:00 **130** ***In vitro*** **transcriptomic analyses of the Aphid's secondary symbiont, *Hamiltonella defense*** - Germain Chevignon, Kerry Oliver, Michael Strand; Department of Entomology, University of Georgia (UGA), Athens, United States

10:15 **131** **A diverse array of new viral sequences identified in worldwide populations of the Asian citrus psyllid (*Diaphorina citri*) using viral metagenomics** - Shahideh Nouri[1], Nida Salem[2], Jared Nigg[1], Bryce Falk[1]; 1University of California (UC Davis), United States; [2]The University of Jordan, Amman, Jordan

CONTRIBUTED PAPERS 08:30-10:30 - *Vouvray*

Microbial control 2

Nina Jenkins

08:30 **132** **The chemical inactivation of H. armigera nucleopolyhedrovirus (HearNPV) on chickpea (*Cicer arietinum*) and other legume crops with studies of its phytochemical mechanism on chickpea** - Aliyu Aminu, Phillip Stevenson, David Grzywacz; Natural Resources Institute, University of Greenwich, Chatham, Kent, United Kingdom

08:45 **133** **Temperature effects on time-to-death, *in vivo* production and insecticidal activity of Agrotis ipsilon baculovirus** - Robert Behle; United States Department of Agriculture, Agricultural Research Service (USDA-ARS), Illinois, United States

09:00 **134** **Controlling false codling moth in citrus with a novel Alphabaculovirus, Cryptophlebia peltastica NPV, and a dual isolate Betabaculovirus preparation, Cryptophlebia leucotreta GV** - Sean Moore; Citrus Research International (CRI), Humewood, South Africa

09:15 **135** **Characterization of Helicoverpa armigera nucleopolyhedrovirus in Brazil** - Fernando Valicente, Victor Costa, Marcus Soares, Francisco Dimate, Fabrício Morgado, Bergmann Ribeiro; Embrapa Maize and Sorghum, Sete Lagoas, MG, Brazil

09:30 **136-STU** **Effect of the Chrysodeixis chalcites single nucleopolyhedrovirus (ChchSNPV) chitinase upon the insecticidal activity of several other alphabaculoviruses** - Eduardo Aguirre[1,2], Oihane Simón[2], Trevor Williams[3], Primitivo Caballero[1,2]; [1]Departamento de Producción Agraria, Universidad Pública de Navarra (UPNA), Pamplona, Spain; [2]Instituto de Agrobiotecnología – CSIC-UPNA, Mutilva, Spain; [3]Instituto de Ecología AC, Xalapa, Veracruz, Mexico

09:45 **137-STU** **Alphabaculovirus of *Mamestra brassicae* (Lepidoptera: Noctuidae): Insecticidal activity against several lepidopteran pests** - Isabel M. Belda[1,2], Maite Arrizubieta[1], Ines Beperet[1], Trevor Williams[3], Primitivo Caballero[1,2]; [1]Departamento de Producción Agraria, Universidad Pública de Navarra (UPNA), Pamplona, Spain; [2]Instituto de Agrobiotecnología – CSIC-UPNA, Mutilva, Spain; [3]Instituto de Ecología AC, Xalapa, Veracruz, Mexico

10:00 **138** **Baculovirus synergism: investigating mixed alphabaculovirus and betabaculovirus infections in the false codling moth, *Thaumatotibia leucotreta*, for improved pest control** - Michael Jukes[1], Caroline Knox[1], Martin Hill[1], Sean Moore[2], Lukasz Rabalski[3], Boguslaw Szewczyk[3]; [1]Rhodes University (RU), Grahamstown, South Africa; [2]Citrus Research International (CRI) Humewood, Port Elizabeth, South Africa; [3]Department of Recombinant Vaccines, Intercollegiate Faculty of Biotechnology, University of Gdansk and Medical University of Gdansk, Poland

10:15 **VI-14-STU** **Genetic and biological characterisation of a novel alphabaculovirus for the microbial control of Cryptophlebia peltastica** - Tamryn Marsberg[1], Martin Hill[1], Caroline Knox[2], Sean Moore[3], B Szewczyk[4], Lukasz Rabalski[4]. [1] Department of Zoology and Entomology, Grahamstown, South Africa; [2] Department of Microbiology and Biotechnology, Grahamstown, South Africa; [3] Citrus Research International, Humewood, Port Elizabeth, South Africa; [4] Department of Molecular Virology, Intercollegiate Faculty of Biotechnology and Medical University of Gdansk, Poland

10:30-11:00 **COFFEE BREAK** *Agnès Sorel*

10:30-13:00 - *Agnès Sorel*

POSTER SESSION

Posters should be displayed

from Monday morning until 2pm Thursday

12:00-13:30 **COCKTAIL LUNCH** *Agnès Sorel*

12:00-13:30 **ICTV meeting** *Bourgueil*

Baculovirus and Nudivirus Study Group

BACTERIA DIVISION SYMPOSIUM 13:30-15:30 - *Chinon*

Unity and diversity of Entomopathogenic bacteria

Sophie Gaudriault & David Clarke

13:30 **140** **Insect Pathogenicity Determinants of Plant-Associated Pseudomonads** - Christoph Keel[1], Peter Kupferschmied[1], Maria Péchy-Tarr[1], Céline Terrettaz[1], Pascale Flury[2], María Pilar Vesga Aguado[2], Monika Maurhofer[2]; [1]University of Lausanne (UNIL) – Department of Fundamental Microbiology, Lausanne, Switzerland; [2]Swiss Federal Institute of Technology Zurich Plant Pathology Group, Institute for Integrative Biology, Zurich, Switzerland

13:55 **141** ***Photorhabdus*** **toxins affecting the cytoskeleton** - Klaus Aktories; Institute of Experimental and Clinical Pharmacology and Toxicology, University of Freiburg, Germany

14:20 **142** ***Photorhabdus*** **Virulence Cassettes: A nano-syringe based toxin secretion and delivery system** - Joseph Healey[1], Guowei Yang[2], Isabella Vlisidou[3], Alexia Hapeshi[1], Nick Waterfield[1]; [1]Division of Biomedical Sciences. Microbiology and Infection unit. Warwick University Medical School, Coventry, United Kingdom; [2]Beijing Pathogen Institute (BPI), China; [3]Bristol University, Life Sciences department., Bristol, United Kingdom

14:45 **143** **Comparative genomics in the entomopathogenic genus *Xenorhabdus*: insight into the XaxAB binary cytolysin-encoding locus** - Gaëlle Bisch, Jean-Claude Ogier, Sylvie Pages, Anne Lanois, Alain Givaudan, Sophie Gaudriault; Diversité, Génomes Interactions Microorganismes – Insectes, INRA, UMR1333, Université de Montpellier, France

15:05 **144** **Virulence determinants of the beepathogenic species *Paenibacillus larvae*** - Elke Genersch; Institute for Bee Research (LIB), Hohen Neuendorf, Germany

CONTRIBUTED PAPERS 13:30-15:30 - *Bourgueil*

Fungi 2

Stefan Jaronski & Jørgen Eilenberg

13:30 **145** **Crude extracts secreted by entomopathogenic mitosporic ascomycetes show potencial for *Ceratitis capitata* (Widemann) (Diptera; Tephritidae) and *Drosophila suzukii* (Matsumura) (Diptera; Drosophilidae) control** - Maria Fernandez-Bravo, Inmaculada Garrido-Jurado, Meryeme El-Betar, Elodie Romero, Meelad Yousef, Enrique Quesada-Moraga; University of Córdoba, Córdoba, Spain

13:45 **146-STU** ***Agrobacterium tumefaciens*****-mediated transgenic *Beauveria bassiana* JEF-007 with reduced virulence against bean bug** - Sihyeon Kim, Se Jin Lee, Yi-Ting Yang, Jae Su Kim; Chonbuk National University, South Korea

14:00 **147** **Virulence of commercial strains of *Beauveria bassiana* and *Metarhizium brunneum* against walnut twig beetle adults and impact on brood production** - Louela Castrillo[1], John Vandenberg[2], Michael Griggs[2], Robert Camp[3], Bryan Mudder[4], Adam Taylor[3], Albert Mayfield[4]; [1]Department of Entomology, Cornell University, Ithaca, United States; [2]USDA ARS, Robert W. Holley Center for Agriculture and Health, Ithaca, United States; [3]Department of Forestry, Wildlife and Fisheries, University of Tennessee, Knoxville, United States; [4]USDA Forest Service – Southern Research Station, Asheville, United States

14:15 **148** **Strategic approach in application of fungal biopesticides: Ecological Biocontrol** - Jae Su Kim, Se Jin Lee[1], Sihyeon Kim[1], Mi Rong Lee[1], Jong Cheol Kim[1], Taek Su Shin[2], Tae Hoon Kim[2]; [1]Department of Agricultural Biology, College of Agricultural & Life Sciences, Chonbuk National University, Jeonju, Korea;

[2]Crop Protection R&D Center, Farm Hannong (LG Affiliated Co.), Nonsan, Korea

14:30 **149-STU** **Natural occurrence of entomopathogenic fungi in apple orchards in Germany related to cropping system and region and evaluation of their efficacy for biocontrol of *Cydia pomonella* -** Carina Ehrich[1], Jessica Reuscher[2], Dietrich Stephan[1]; [1]Julius Kühn-Institut, Institute for Biological Control, Darmstadt, Germany; [2]Frankfurt University of Applied Sciences, Germany

14:45 **150** **Characterization and virulence of *Beauveria bassiana* associated with auger beetle (*Sinoxylon anale*) infesting *Pimenta dioica* -** Senthil Kumar C M, Jacob T K, Devasahayam S, Sharon D'Silva, Nandeesh P G; ICAR-Indian Institute of Spices Research, Kozhikode, Kerala, India

15:00 **151-STU** **Involvement of Tenecin3 in the infection process of *Tenebrio molitor* by *Beauveria bassiana*** - Sevasti Maistrou, Nicolai Meyling, Annette Jensen, Caroline Zanchi; Department of Plant and Environmental Sciences, University of Copenhagen, Frederiksberg, Denmark

15:15 **121-STU** **The influence of orchard age on entomopathogenic fungi and nematode population dynamics -** Sonnica Albertyn[1], Martin Hill[1], Moore Sean; [1]Rhodes University, Department of Zoology and Entomology, Grahamstown, South Africa; [2]Citrus Research International, Port Elizabeth, South Africa

CONTRIBUTED PAPERS 13:30-15:30 - *Vouvray*

Virus 5

Ikbal Agah Ince & Sassan Asgari

13:30 **152-STU** **Interactions between the salivary gland hypertrophy virus and its host immune system -** Irene Meki[1,2], Ikbal Ince[3], Henry Kariithi[4], Drion Boucias[5], Just Vlak[1], Monique Van Oers[1], Adly Abd-Alla[2]; [1]Laboratory of Virology, Wageningen University, Netherlands; [2]Insect Pest Control Laboratories, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna, Austria – Austria; [3]Department of Medical Microbiology, Acibadem University, Istanbul, Turkey; [4]Biotechnology Research Institute, Kenya Agricultural Livestock Research Organization, Nairobi, Kenya; [5]Department of Entomology and Nematology, University of Florida, Gainesville, Florida, United States

13:45 **153-STU** **Host range of Glossina pallidipes salivary gland hypertrophy virus (GpSGHV) -** Guler Demirbas Uzel[1,2], Andrew Parker[1], Robert Mach[2], Adly Abd-Alla[1]; [1]International Atomic Energy Agency - Insect Pest Control Laboratory (IAEA IPCL), Austria; [2]Vienna University of Technology, Vienna, Austria

14:00 **154** **Highjack of intracellular signalling pathways and robust immune responses explain the hytrosavirus-induced differential pathologies in two *Glossina* model species -** Ikbal Agah Ince[1], Henry Kariithi[2], Sjef Boeren[3], Irene Meki[4,5], Edwin Murungi[6], Everlyne Otieno6, Steven Ger Nyanjom[6], Monique Van Oers[4], Just M. Vlak[4], Adly Abd-Alla[5]; [1]Acibadem University, Department of Medical Microbiology, School of Medicine, Atasehir, Turkey; [2]Biotechnology Research Institute, Kenya Agricultural and Livestock Research Organization, Nairobi, Kenya ; [3]Laboratory of Biochemistry, Wageningen University, Netherlands; [4]Laboratory of Virology, Wageningen University, Netherlands; [5]Insect Pest Control Laboratories, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna, Austria; [6]Department of Biochemistry, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya

14:15 **155** **The salivary gland proteome of *Glossina m. morsitans*, parasitized with *Trypanosoma b. brucei* -** Henry Kariithi[1], Sjef Boeren[2], Just M. Vlak[3], Adly Abd-Alla[4]; [1]Biotechnology Research Institute, Kenya Agricultural and Livestock Research Organization, Nairobi, Kenya; [2]Laboratory of Biochemistry, Wageningen University, Netherlands; [3]Laboratory of Virology, Wageningen University, Netherlands; [4]Insect Pest Control Laboratories, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna, Austria

14:30 **156** **Characterization of Bustos virus, a new member of the *Negevirus* group isolated from a *Mansonia* mosquito in the Philippines -** Ryosuke Fujita[1,2], Ryusei Kuwata[3], Daisuke Kobayashi[1], Arlene Bertuso4, Haruhiko Isawa[1], Kyoko Sawabe[1]; [1]National Institute of Infectious Diseases, Japan; [2]Japan Agency for Medical Research and Development, Japan; [3]Yamaguchi University, Japan; [4]University of the Philippines Manila, Philippines

14:45 **157** **RNA activation in mosquito cells and its suppression by the dengue virus NS5 protein -** Sultan Asad, Mazhar Hussain, Sassan Asgari; The University of Queensland, School of Biological Sciences, Brisbane, Australia

15:00 **158** **Zika virus epidemic in Americas -** Julien Thézé, Nuno Rodrigues Faria, Oliver Pybus; Department of Zoology, University of Oxford, United Kingdom

15:15 **159-STU** **New RNA virus producing covert infections in field and laboratory insects of *Ceratitis capitata* (Wiedemann) -** Angel Llopis-Giménez[1,2], Rosa Ma González-Martínez[1,2], Anabel Millán-Leiva[3], Elena Llácer[4], Marta Catalá[4], Alberto Urbaneja[4], Salvador Herrero[1,2]; [1]Departamento de Genetica, Universitat de Valencia, Spain; [2]Estructura de Recerca Interdisciplinar en Biotecnologia i Biomedicina, Universitat de Valencia, Spain; [3]Instituto de Hortofruticultura Subtropical y Mediterranea "La Mayora", CSIC, Malaga, Spain; [4]Instituto Valenciano de Investigaciones Agrarias, Centro de Proteccion Vegetal y Biotecnologia, Unidad Asociada de Entomologia, Moncada, Spain

CONTRIBUTED PAPERS 13:30-15:30 - *Courteline*

Microbial control 3

Sean Moore

13:30 **160-STU** **Whole Genome Sequencing of PhopGV Isolates for Control of *Tuta absoluta* in Tomato and *Phthorimaea operculella* and *Tecia solanivora* in Potato -** Andreas Larem, Eva Fritsch, Karin Undorf-Spahn, Jörg Wennmann, Johannes Jehle; Federal Research Centre for Cultivated Plants, Institute for Biological Control, Darmstadt, Germany

13:45 **161-STU** **Identification of a novel mode of resistance against Cydia pomonella granulovirus in codling moth indicates a highly dynamic adaptation in the host population -** Annette Sauer[1], Petr Nguyen[2], Eva Fritsch[1], Karin Undorf-Spahn[1], Kento Iwata[3], Madoka Nakai[3], David Heckel[4], Frantisek Marec[2], Johannes Jehle[1]; [1]Julius Kühn Institut, Germany; [2]Biology Centre, Czech Republic; [3]Tokyo University of Agriculture and Technology, Japan; [4]Max Planck Institute for Chemical Ecology, Germany

14:00 **162** **Entomopathogenic fungi as control agents of *Thaumatotibia leucotreta* in citrus orchards: efficacy and persistence -** Candice Coombes[1], Martin Hill[1], Sean Moore[2], Joanna Dames[3]; [1]Department of Zoology and Entomology, Rhodes University, Grahamstown, South Africa; [2]Citrus Research International, Port Elizabeth, South Africa; [3]Department of Biochemistry and Microbiology, Rhodes University, Grahamstown, South Africa

14:15 **163** **Control of wireworms in organic potato farming is feasible with an attract-and-kill strategy: technical aspects -** Anant Patel, Stefan Vidal, Mario Schumann, Wilhelm Beitzen-Heineke, Marina Vemmer; Bielefeld University of Applied Sciences, Department of Engineering Sciences and Mathematics, Bielefeld, Germany

14:30 **164** **Control of wireworms in organic potato farming is feasible with an attract-and-kill strategy: field trials and mantraps on the way to registration -** Stefan Vidal, Anant Patel, Marina Vemmer, Wilhelm Beitzen-Heineke, Mario Schumann; Department of Crop Sciences, University of Göttingen, Germany

14:45 **165-STU** **Screen bag formulations (SBF) of entomopathogenic *Beauveria* and *Metarhizium* conidia from granular substrates to control *Riptortus pedestris* -** Se Jin Lee, Sihyeon Kim, Mi Rong Lee, Jae Su Kim; Chonbuk National University, South Korea

15:00 **166-STU** **Entomopathogenic fungal library to control *Locusta migratoria* in Korea** - Mi Rong Lee, Se Jin Lee, Sihyeon Kim, Jong Cheol Kim, Jae Su Kim†; Chonbuk National University, South Korea

15:15 **167-STU** **Ambrosia beetle mortality and reduced brood production following exposure to microbial control fungi** - Louela Castrillo[1], Michael Griggs[2], John Vandenberg[2]; [1]Dept of Entomology, Cornell University, Ithaca, United States; [2]USDA ARS, Robert W. Holley Center for Agriculture and Health, Ithaca, United States

15:30-16:00 **COFFEE BREAK** *Agnès Sorel*

NEMATODE DIVISION SYMPOSIUM 16:00-18:00 - *Chinon*

Harnessing Metabolites from Entomopathogenic Nematode Symbiotic Bacteria for Broad Use

David Shapiro-Ilan & Selcuk Hazir

16:00 **168** **The regulation of secondary metabolism and natural**

product production in *Photorhabdus* - Davíd Clarke; University College Cork, Ireland

16:30 **169 Identification and application of eicosanoid biosynthesis inhibitors synthesized by *Xenorhabdus* and *Photorhabdus* -** Yonggyun Kim; Andong National University, South Korea

17:00 **170 Using *Photorhabdus* and *Xenorhabdus* metabolites for control of pecan and peach diseases -** Selcuk Hazir[1], Clive Bock[2], David Shapiro-Ilan[2]; [1]Adnan Menderes University Faculty of Arts and Sciences Department of Biology, Turkey; [2]USDA-ARS, Southeastern Fruit and Tree Nut Research Laboratory Byron, United States

17:30 **171 Natural products from entomopathogenic bacteria - from bugs to the clinic?** Helge Bode**;** Goethe-University Frankfurt am Main, Merck endowed Chair for Molecular Biotechnology, Dept of Biosciences, Frankfurt am Main, Germany

CONTRIBUTED PAPERS 16:00-18:00 - *Courteline*

Microbial control 4

Dietrich Stephan

16:00 **172 Synergistic combinations of an emulsifiable formulation of *Beauveria bassiana* and a pyrethroid insecticide against insecticide-resistant annual bluegrass weevil, *Listronotus maculicollis*, adults -** Shaohui Wu, Albrecht Koppenhofer, Olga Kostromytska; Department of Entomology, Rutgers University, New Brunswick, United States

16:15 **173 Residual efficacy of *Beauveria bassiana* (Balsamo) Vuillemin, diatomaceous earth, Imidacloprid against three Coleopteran and one psocid species of stored grains -** Waqas Wakil[1], Thomas Schmitt[2]; [1]Department of Entomology, University of Agriculture, Faisalabad, Pakistan; [2]Senckenberg German Entomological Institute, Müncheberg, Germany; Institute of Zoology, Faculty of Natural Sciences I, Martin-Luther-University Halle-Wittenberg, Germany; Dept of Biogeography, Faculty of Regional and Environmental Sciences, Trier University, Germany

16:30 **174-STU Biological Efficacy of the Entomopathogenic Fungi *Isaria fumosorosea* as a Biocontrol Agent Against Pest Insects** Katharina Saar[1], Jasmin Philippi[2], Edgar Schliephake[2], Nicolas Maguire[3], Johannes Jehle[1], Dietrich Stephan[1]; [1]Julius Kühn-Institute, Darmstadt, Germany; [2]Julius Kühn Institut, Quedlinburg, Germany; [3]University of Applied Science, Department of Life Science Engineering, Gießen, Germany

16:45 **175 Fungal entomopathogens as endophytes for plant protection: Can they promote plant growth as well? -** Lara R. Jaber[1], Juerg Enkerli[2]; [1]Department of Plant Protection, Faculty of Agriculture, University of Jordan, Amman, Jordan; [2]Agroscope, Zurich, Switzerland

17:00 **176 Mortality, fecundity and behavior of *Aphis gossypii* Glover feeding on melon leaves endophytically colonized by entomopathogenic fungi -** Natalia Gonzalez Mas, Enrique Quesada Moraga; Agricultural and Forestry Sciences, ETSIAM, University of Córdoba. Campus de Rabanales, Córdoba, Spain

17:15 **236-STU Non-target effects of *Metarhizium brunneum* on microbial communities assessed in pot and field trials to control *Agriotes* spp** - Johanna Mayerhofer[1], Sonja Eckard[1], Martin Hartmann[2], Giselher Grabenweger[1], Adrian Leuchtmann[3], Franco Widmer[1], Jürg Enkerli[1]; [1]Institute for Sustainability Sciences Agroscope, Zurich, Switzerland; [2]Swiss Federal Institute for Forest, Snow and Landscape Research WSL, Birmensdorf, Switzerland; [3]Plant Ecological Genetics, ETH Zurich, Switzerland

CONTRIBUTED PAPERS 16:00-18:00 - *Bourgueil*

Disease of Beneficial Invertebrates 2

Helen Hesketh

16:00 **177 Entomopathogens and contaminant microbes of insects for food and feed value chain in Africa -** Subramanian Sevgan, Fiaboe Komi, Ekesi Sunday; International Centre of Insect Physiology and Ecology (icipe), Nairobi, Kenya

16:15 **178 Implications of the honeybee microbial community in the response to major parasites and pathogens** - Maria Giovanna Marche[1], Ignazio Floris[1], Alberto Satta[1], Luca Ruiu[1,2]; [1]Department of Agriculture, University of Sassari, Italy; [2]Bioecopest Srl, Technology Park of Sardinia, Tramariglio, Italy

16:30 **179 First detection of the Apis mellifera filamentous virus (AmFV) in honey bees (*Apis mellifera*) in China -** Chunsheng Hou, Beibei Li, Shuai Deng, Yuexiong Luo, Qingyun Diao; Institute of Apicultural Research, Chinese Academy of Agricultural Sciences, China

16:45 **180 Trans-generational immune priming in *Tenebrio molitor*: towards the identification of the molecular mechanisms -** Guillaume Tetreau[1], Julien Dhinaut[2], Philippe Bulet[3], Yannick Moret2, Benjamin Gourbal[1]; [1]CNRS-IFREMER : UMR5244, Université de Perpignan, Université de Montpellier, Organisation mondiale de la santé (OMS/WHO), Perpignan, France, [2]Laboratoire Biogéosciences, CNRS UMR6282, Université de Bourgogne, Dijon, France; [3]Laboratoire Andrologie Gérontechnologie Inflammation Modélisation, CNRSFRE3405, Université Joseph Fourier, Grenoble I, France; Platform Biopark Archamps, France,

17:00 **181 Molecular cloning and prokaryotic expression of RdRp gene of IAPV -** Shuai Deng, Qingyun Diao, Beibei Li, Chunsheng Hou*;* Institute of Apicultural Research, Chinese Academy of Agricultural Sciences, China

CONTRIBUTED PAPERS 16:00-18:00 - *Vouvray*

Virus 6

Trevor Williams & Martin Erlandson

16:00 **182 Genotype co-occlusion as a novel paradigm for the development of virus-based insecticides: is the evidence sufficiently convincing yet?** - Primitivo Caballero[1,2], Ines Beperet1, Oihane Simon[1], Maite Arrizubieta[1], Miguel Lopez-Ferber[3], Trevor Williams[4]; [1]Instituto de Agrobiotecnología, CSIC-UPNA, Mutilva, Spain; [2]Departamento de Producción Agraria, Universidad Pública de Navarra, Pamplona, Spain; [3]Ecole des Mines d'Alès, France; [4]Instituto de Ecología AC, Xalapa, Mexico

16:15 **183 Improving infectivity of baculovirus by high-efficiently embedding the enhancing factors into occlusion bodies -** Shili Yang, Ruipeng Ma, Lijuan Zhao, Jia Hu, Chengfeng Lei, Xiulian Sun*;* Wuhan Institute of Virology, Chinese Academy of Sciences, China

16:30 **184 Baculovirus efficacy against the fall armyworm varies with intraspecific genetic variation in soybean defence traits -** Ikkei Shikano[1], Ketia Shumaker[2], Michelle Peiffer[1], Gary Felton[1], Kelli Hoover[1]; [1]Department of Entomology and Center for Chemical Ecology, Pennsylvania State University, United States; [2]Department of Biological and Environmental Sciences, University of West Alabama, United States

16:45 **185 Genetic and biological characterisation of anovel South African Cydia pomonella granulovirus (CpGV-SA) with potential for use in resistance management strategies -** Caroline Knox[1], Boitumelo Motsoeneng[1], Martin Hill[1], Sean Moore[2]; [1]Rhodes University, South Africa; [2]Citrus Research International (CRI), South Africa

17:00 **186 Baculovirus isolated from *Lymantria dispar* larvae as an example of possible virus adaptation to a new host -** Lukasz Rabalski[1], Martyna Krejmer-Rabalska[1], Iwona Skrzecz[2], Boguslaw Szewczyk[1]; [1]Laboratory of Recombinant Vaccines, Intercollegiate Faculty of Biotechnology, University and Medical University, Gdansk, Poland; [2]Forest Research Institute, Sekocin Stary, Poland

17:15 **187 Genomics of alphabaculovirus isolates infecting *Mamestra* species from North America and Eurasia -** Martin Erlandson1, Doug Baldwin[1], Just Vlak[2], David Theilmann[3]; [1]Saskatoon Research and Development Centre, AAFC, Canada; [2]Laboratory of Virology, Wageningen University, Netherlands; [3]Summerland Research and Development Centre, AAFC, Canada

17:30 **188 Improved insecticidal activity of Chilo iridescent virus expressing an insect specific neurotoxin** - Remziye Nalcacioglu[1], Hacer Muratoglu[1], Aydin Yesilyurt[1], Arzu Ozgen[1], Zihni Demirbag[1], Van Oers Monique[2], Vlak Just[2]; [1]Karadeniz Technical University, Faculty of Science, Department of Biology, Turkey; [2]Wageningen University, Netherlands

17:45 **189 Characterization of the Baculovirus-Densovirus interaction when co-infecting the same host -** Laila Gasmi[1], Mylène Ogliastro[2], Salvador Herrero[1]; [1]Laboratory of Biotechnological Pest Control, Department of Genetics, and Estructura de Recerca Interdisciplinar en Biotecnologia i Biomedicina, Universitat de València, Spain; [2]Laboratory DGMI, UMR 1333, Université Montpellier II, France

18:00-19:45

Division Business Meetings and Workshops

Microbial Control Division Meeting *Courteline*
Nematode Division Meeting *Chinon*
'Shooting a worm: insights on nematode photography'
J. Eisenback
DBI Division Meeting *Bourgueil*
'Coral Diseases' - M. Sweet
Virus Division Meeting *Vouvray*
'*Taxonomy of Polydnaviruses'* **-** M. Strand

THURSDAY – 28 July

07:00-08:00 **Qi Gong** *Foyer*
08:00-12:00 **Registration** *Hall*

VIRUS DIVISION SYMPOSIUM 08:00-10:00 - *Descartes*

Viruses and horizontal gene transfers

Elisabeth Herniou & Jean-Michel Drezen

08:00 **190** **Mechanisms of horizontal genes transfer in Metazoans** - Chiara Boschetti, Isobel Eyres, Alastair Crisp, Elton Gargioni Grisoste Barbosa, Timothy Barraclough, Gos Micklem, Alan Tunnacliffe, Department of Chemical Engineering and Biotechnology, University of Cambridge, United Kingdom

08:20 **191** **Evidence of recent interspecies horrizontal genes transfer regarding nucleopolyhedrovirus infection of *Spodoptera frugiperda*** - Mariano Belaich[1], Gloria Barrera[2], Manuel Patarroyo[3], Laura Villamizar[2], Pablo Ghiringhelli[1], [1]Universidad Nacional de Quilmes, Laboratorio de Ingeniería Genética y Biología Celular y Molecular, Bernal, Argentina; [2]Corporación Colombiana de Investigación Agropecuaria, Cundinamarca, Colombia; [3]Fundación Instituto de Inmunología de Colombia, Bogotá, Colombia

08:40 **192** **Continuous influx of genetic material from host to virus populations -** Gilbert Clément[1], Jean Peccoud[1], Aurélien Chateigner[2], Bouziane Moumen[1], Richard Cordaux[1], Elisabeth Herniou[2]; [1]UMR CNRS 7267 Ecologie et Biologie des Interactions, Equipe Ecologie Evolution Symbiose, Université de Poitiers, Poitiers, France; [2]Institut de recherche sur la Biologie de l'Insecte, CNRS UMR 7261, Université François-Rabelais, Tours, France

09:00 **193** **Microplitis demolitor bracovirus DNAs integrate into the genome of host cells** - Michael Strand, Department of Entomology, University of Georgia, Athens, United States

09:20 **194** **Acquisition and Domestication of bracoviral genes in *Spodoptera* spp contributes to their defense against pathogens** - Laila Gasmi[1], Jean-Michel Drezen[2], Salvador Herrero[1]; [1]Laboratory of Biotechnological Pest Control, Department of Genetics, and Estructura de Recerca Interdiciplinar en Biotecnologia i Biomedicina; [2]Institut de recherche sur la Biologie de l'Insecte, CNRS UMR 7261, Université François-Rabelais, Tours, France

CONTRIBUTED PAPERS 08:00-10:00 - *Courteline*

Fungi 3

Nicolai Vitt Meyling & Annette Bruun Jensen

08:00 **195** **Molecular characterization of icipe EPF isolates: opportunities and challenges** - Fathiya Khamis, Nguya Maniania, Komivi Akutse, Levi Ombura, Subramanian Sevgan, Sunday Ekesi; International Center of Insect Physiology and Ecology (ICIPE) – Kenya

08:15 **196** **The comparative analysis of defense reactions and midgut microbiota of *Galleria mellonella* under development of mycoses caused by *Metarhizium robertsii* and *Ordyceps militaris* -** Olga Yaroslavtseva[1], Oksana Tomilova[1], Alex Pervushin[2], Natalia Kryukova[1], Ecatherine Chertkova[1], Olga Polenogova[1], Maxim Tyurin[1], Ivan Dubovskiy[1], Viktor Glupov[1], Vadim Kryukov; [1]Institute of Systematics and Ecology of Animals, Siberian Branch of Russian Academy of Sciences, Russia; [2]All-Russian Research Institute of Plant Protection, Russia

08:30 **197-STU** **The histone deacetylase HosA regulates cell cycle, conidiation, virulence and stress tolerance in *Beauveria bassiana*** - Qing Cai, Sheng-Hua Ying, Ming-Guang Feng; Zhejiang University, Hangzhou, Zhejiang Province, China

08:45 **198-STU** **A novel vacuolar protein is required for the *in vitro* asexual cycle and full virulence of *Beauveria bassiana***; Zhenjian Chu, Sheng-Hua Ying, Ming-Guang Feng; Zhejiang University, Hangzhou, Zhejiang Province, China

09:00 **199-STU** **Characterization of high osmolarity glycerol pathway essential for environmental adaptation in *Beauveria bassiana*;** Jing Liu, Sheng-Hua Ying, Ming-Guang Feng Zhejiang; University, Hangzhou, Zhejiang Province, China

CONTRIBUTED PAPERS 08:15-10:00 - *Bourgueil*

Diseases of Beneficial Invertebrate 3

Grant Stentiford

08:15 **200** **Applications of environmental DNA (eDNA) methods in parasitology** - David Bass[1,2], Georgia Ward[3], Rose Kerr[1], Catherine Troman[2], Corey Holt[1], Kelly Bateman[1], Beth Okamura[2], Grant Stentiford[1]; [1]European Union Reference Laboratory for Crustacean Diseases and/or Centre for Environment, Fisheries and Aquaculture Science (Cefas), Weymouth, United Kingdom; [2]The Natural History Museum – United Kingdom

08:30 **201** **Exploring into an emerging disease in the circum-Antarctic keystone predator, the sea star *Odontaster validus*** - Laura Núñez Pons[1,2,3], Thierry Work[4], Robert Rameyer[4], Juan Moles[4], Carlos Angulo-Preckler[3], Conxita Avila; [1]Smithsonian Tropical Research Institute (STRI), Panama; [2]Hawai Institute of Marine Biology (HIMB), University of Hawaii at Manoa, Hawaii, United States; [3]Universitat de Barcelona Barcelona, Spain; [4]US Geological Survey, National Wildlife Health Center, Honolulu, United States

202-STU Cancelled

08:45 **203-STU** **Impact of water temperature on immune-related gene expression in American lobster experimentally infected with White Spot Syndrome Virus** - Louise-Marie Roux[1,2], Philip Byrne[2], Fraser Clark[1], Spencer Greenwood; [1]Dept of Biomedical Sciences and Lobster Science Centre, Atlantic Veterinary College, University of Prince Edward Island, Canada; [2]Gulf Biocontainment Unit-Aquatic Animal Health Laboratory, Fisheries and Oceans Canada, Canada; [3]Dept of Pathology and Microbiology, Atlantic Veterinary College, University of Prince Edward Island, Canada

09:00 **204-STU** **Development of a duplex PCR as screening tool for the detection of Crangon crangon bacilliform virus in the European brown shrimp *Crangon crangon*** - Benigna Van Eynde[1,2], Olivier Christiaens[1], Daan Delbare[2], Kelly Bateman[3], Grant Stentiford[3], Annette Dullemans[4], Monique Van Oers[5], Guy Smagghe[1]; [1]Ghent University, Ghent, Belgium; [2]Institute of Agricultural and Fisheries Research, Ostend, Belgium; [3]European Union Reference Laboratory for Crustacean Diseases, CEFAS, Weymouth, United Kingdom; [4]Wageningen University and Research Center Wageningen, Netherlands

09:15 **205** **Microbial patters allied to coral disease and bleaching, insights from Kane'Ohe Bay – Oahu (Hawaii) -** Laura Núñez Pons[1,2,3], Raphael Ritson-Williams[2], Emilia Sogin[2], Ross Cunning[2], Anthony Amend[2]; [1]Smithsonian Tropical Research Institute, Panama; [2]Hawai Institute of Marine Biology, University of Hawaii at Manoa, Hawaii, United States; [3]Universitat de Barcelona, Barcelona, Spain

CONTRIBUTED PAPERS 08:15-10:00 - *Vouvray*

Microbial Control 5

Ben Raymond

08:15 **206-STU** **Controlling Invasive Crustacea** - Jamie Bojko[1], Alison Dunn[2], Paul Stebbing[1], Grant Stentiford[1]; [1] CEFAS, Weymouth, Dorset, United Kingdom; [2]University of Leeds, United Kingdom

08:30 **207** **Molecular characterization of the plasmid-encoded Pir-like binary toxins isolated from shrimp suffering acute hepatopancreatic necrosis disease or early mortality syndrome (EMS/AHPND)** - Kallaya Sritunyalucksana, Jiraporn Srisala, Suparat Taengchaiyaphum, Anuphap Prachumwat, **Ornchuma Itsathitphaisarn,** Timothy Flegel; Shrimp-Virus Interaction Laboratory, National Center for Genetic Engineering and Biotechnology, Bangkok, Thailand

208 Cancelled

08:45 **209** **DEBtox modelling of pathogen-mortality data over time; a novel toxicokinetic-toxicodynamic approach to derive dose**

effects - Helen Hesketh, Jan Baas; Centre for Ecology Hydrology, Wallingford, United Kingdom

09:00 **210 A putative esterase is involved in toxicity of the mexican strain *Serratia entomophila* Mor4.1 towards larvae of *Phyllophaga* Spp (Coleoptera)** - María Nunẽz-Valdez, Manuel Martínez-Tapia, Jianwu Chen, Sarjeet Gill; Centro de Investigación en Dinámica Celular, Instituto de Investigación en Ciencias Básicas y Aplicadas, Universidad Autónoma del Estado de Morelos, Cuernavaca Morelos, Mexico

09:15 **211-STU Monitoring and expression analysis of *Pseudomonas* protegens CHA0 during colonization of Lepidoptera** - María Del Pilar Vesga Aguado[1], Pascale Flury[1], Monika Maurhofer[1], Christoph Keel[2]; [1]Swiss Federal Institute of Technology, Zurich, Switzerland; [2]Université de Lausanne, Lausanne, Switzerland

10:00-10:30 **COFFEE BREAK** *Agnès Sorel*

10:30 - 12:30 - *Descartes*

SIP Business Meeting

12:30-14:00 **BUFFET LUNCH** *Agnès Sorel*

12:30-14:00 **Student Competition Jury** *Bourgueil*

MICROSPORIDIA DIVISION SYMPOSIUM 14:00-16:00 - *Bourgueil*

Host Pathogen interactions

Susan Bjornson

14:00 **212 Inhibition of apoptosis is a universal mechanism for intracellular survival of microsporidia?** - Yuliya Sokolova[1,2], Xavier Alvarez[3], Lisa Bowers[3], Elizabeth Didier[3]; [1]Institute of Cytology, Russian Academy of Sciences, St Petersburg, Russia; [2]Louisana State University School of Veterinary Medicine, Baton Rouge LA, United States; [3]Tulane National Primate Research Center, Covington LA, United States

14:**30** **213 Mosquito-Microsporidia Model Systems for Understanding Morphological and Phylogenetic Relationships** James Becnel; U.S. Department of Agriculture Agricultural Research Service, Gainesville, Florida, United States

15:00 **214 Pathogenicity, prevalence and intensity of a microsporidian infection by *Nosema fumiferanae* postvittana subsp. n. in the light brown apple moth, *Epiphyas postvittana*, in California** - Julie Hopper[1,2], Wei-Fone Huang[3,4], Leellen Solter[3], Nicholas Mills[1]; [1]University of California, Berkeley, United States; [2]University of California, Davis, United States, [3]University of Illinois, Urbana-Champaign, United States, [4]College of Bee Science, Fujian Agriculture and Forestry University, China

15:30 **215 Comparative genomics of microsporidia that infect marine organisms** - Bryony Williams; Biosciences, College of Life and Environmental Sciences, University of Exeter, Devon, United Kingdom

CONTRIBUTED PAPERS 14:00-16:00 - *Courteline*

Bacteria 4

Marianne Carey & Shuyuan Guo

14:00 **216 How to eat a Crystal protein: Crystal protein Cry5B as a novel and powerful anti-infective for humans** - Yan Hu, David Koch, Zeynep Mirza, Thanh-Thanh Nguyen, Gary Ostroff, Raffi Aroian; Program in Molecular Medicine, UMASS Medical School, United States

14:15 **217 A biochemical comparison of VIP3Ab1 and VIP3B insecticidal proteins** - Marc Zack, Megan Sopko, Ted Letherer, Sek Yee Tan, Kenneth Narva; Dow AgroSciences, Indianapolis, United States

14:30 **218 Bio-polymer microencapsulations of *Bacillus thuringiensis* crystal preparations for improved longterm larvicidal activity** - He Xiaolin[1], He Kanglai[2], Guo Shuyuan[1]; [1]School of Life Science, Beijing Institute of Technology, Beijing, China; [2]State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China

219 Cancelled

14:45 **220 Enterocyte purge and rapid recovery as a novel reaction of the gut epithelium to toxin or xenobiotics exposur**e - Kwang-Zin Lee[1], Matthieu Lestradet[1], Catherine Socha[1], Stefanie Schirmeier[1], Antonin Schmitz[2], Caroline Spenle[3], Olivier Lefebvre[3], Céline Keime[4], Samuel Liegeois[1], Miriam Yamba[1], Richard Bou Aoun[1], Yannick Schwab[4], Frédéric Dalle[2], Patricia Simon-Assmann[3], Dominique Ferrandon[1,5]; [1]Equipe Fondation Recherche Medicale, CNRS – Strasbourg, France; [2]UMR 1342 University of Burgundy, Dijon, France; [3]MN3T, FMTS, LABEX Medalis University of Strasbourg U1109 INSERM, France; [4]CNRS INSERM Univ. of Strasbourg, IGBMC, France; [5]Institut de Biologie Moleculaire et Cellulaire, CNRS, Strasbourg, France

15:00 **221 Biomphalysin, a novel family of snail immune effectors with common features with bacterial pore-forming toxins** - Silvain Pinaud, Guillaume Tetreau, Anaïs Portet, Richard Galinier, Cristian Chaparro, Benjamin Gourbal, David Duval; Host-Pathogen-Environment Interactions Laboratory, CNRS UMR5244, Université de Perpignan Via Domitia, Université de Montpellier, Institut Français de Recherche pour l'Exploitation de la Mer, OMS/WHO, Perpignan, France

15:15 **222 Toxicological and protein characterization of *Bacillus sphearicus* C3-41 strain from Karnataka, India** -Basavaraj Kalmath[1], Gajanan Katkar[2], Aralimarad Prabhuraj[1], Patil Basavaraj[1]; [1]College of Agriculture, University of Agricultural Science, Raichur, Karnataka, India; [2]Department of Biochemistry, Mysore University, Karnataka, India

CONTRIBUTED PAPERS 14:00-16:00 - *Descartes*

Virus 7

Madoka Nakai & Gary Blissard

14:00 **223 Comparative genomics of parasitoid wasps and what it tells on the evolution of symbiotic viruses** - Jérémy Gauthier[1], Annie Bézier[1], Jean-Marc Aury[2], Valérie Barbe[2], Anthony Bretaudeau[3], Fabrice Legeai[3], Karine Musset[1], Diane Bigot[1], Thibaut Josse[1], Sébastien Moreau[1], Philippe Gayral[1], Elisabeth Huguet[1], Elisabeth Herniou[1], Jean-Michel Drezen[1]; [1]Institut de recherche sur la Biologie de l'Insecte, CNRS UMR 7261, Université François-Rabelais, Tours, France; [2]Centre national de séquençage CEA, Genoscope, Evry, France; [3]Institut de Génétique, Environnement et Protection des Plantes, INRA UMR1349, Rennes, France

14:15 **224 Permissiveness of lepidopteran hosts is linked to differential expression of bracovirus genes** - Kavita Bitra, Gaelen Burke, Michael Strand; [1]University of Georgia, Athens, United States

14:30 **225 Latency-deficient recombinant and mutant Helicoverpa zea nudiviruses that cause enhanced pathology and sterility to their insect hosts** - Bruce Webb, Kendra Steele, Angelika Fath-Goodin, Alonna Wright, Brooke Nemec; University of Kentucky Lexington, United States

14:45 **226 The postfusion 3D-structure of the Spodoptera exigua multiple nucleopolyhedrovirus envelope fusion protein F** - Qiushi Wang[1], Ieva Vasiliauskaite[3], Berend Jan Bosch[2], Thomas Krey[3], Peter Rottier[2], Just M. Vlak [1], Felix Rey[3]; [1]Laboratory of Virology, Wageningen University, Wageningen, Netherlands; [2]Virology Division, Department of Infectious Disease and Immunology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, Netherlands; [3]Structural Virology Unit, Department of Virology, Institut Pasteur, Paris, France

15:00 **227 A new system for studies of viral envelope protein trafficking in insect cells** - Jeffrey Hodgson[1], Nicolas Buchon[2], Gary Blissard[1]; [1]Boyce Thompson Institute at Cornell University, Ithaca, NY, United States; [2]Department of Entomology Cornell University, Ithaca, NY, United States

15:15 **228 Rescue of the entry of AcMNPV fusion-defective mutants by low-pH triggering: higher fusion activity is required for GP64-mediated entry into mammalian cells compared to insect cells?** - Hu Liangbo, Li Yimeng, Ning Yunjia, Deng Fei, Hu Zhihong, Wang Manli, Wang Hualin; State Key Laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, China

15:30 **229 Extra genomic DNA elements found in an entomopoxvirus** - Shusuke Koike[1], Jun Takatsuka[2], Julien Thézé[3], Elisabeth Herniou[4], Madoka Nakai[1]; [1]Tokyo University

of Agriculture and Technology, Fuchu-shi, Tokyo, Japan; [2]Forestry and Forest Products Research Institute, Tsukuba, Japan; [3]University of Oxford, Department of Zoology, Oxford, United Kingdom; [4]Institut de recherche sur la biologie de l'insecte, CNRS UMR7261, Université François Rabelais, Tours, France

CONTRIBUTED PAPERS 14:00-16:00 - *Vouvray*

Microbial Control 6

Mike Brownbridge

14:00 **230** **Entomopathogenic fungi for managing exotic and endemic pests in vegetable crops in California** - Surendra Dara; University of California Cooperative Extension, United States

14:15 **231** **Experimental devices treated with *Metarhizium brunneum* and its extract for spotted-wing drosophila *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) control** - Meelad Yousef, Enrique Aranda-Valera, Inmaculada Garrido-Jurado, Enrique Quesada-Moraga; University of Córdoba, Córdoba, Spain

14:30 **232** **Optimization of a coating process for the development of *Metarhizium*-formulations for control of soil dwelling pest**s - Dietrich Stephan[1], Nicolas Maguire[2]; [1]Institute for Biological Control, Julius Kühn-Institut, Darmstadt, Germany; [2]Technische Hochschule Mittelhessen, Gießen, Germany

14:45 **233** **Physiological mechanisms of synergy between pyrethroid insecticide and entomopathogenic fungus *Metarhizium robertsii* on nontarget aquatic model species *Daphnia magna*** - Yury Noskov[1], Olga Yaroslavtseva[2], Ecatherine Chertkova[2], Vadim Kryukov[2], Ivan Dubovskiy[2]; [1]National Research Tomsk State University, Tomsk, Russia; [2]Institute of Systematics and Ecology of Animals, Siberian Branch of Russian Academie of Sciences, Novosibirsk, Russia

15:00 **234** **Virulence of wild and transformed strains of *Metarhizium anisopliae* ICIPE30 against *Rhipicephalus appendiculatus* and *Amblyomma variegatum* ticks** - Nana Paulin[1,2], Khamis Fathiya[1], Ekesi Sunday[1], Subramanian Sevgan[1], Ombura Levi[1], Maniania Nguya[1]; [1]International Centre of Insect Physiology and Ecology, Nairobi, Kenya; [2]Universite de Dschang, Dschang, Cameroon

15:15 **235** **Field evaluation of the entomopathogenic fungus *Metarhizium anisopliae* for the control of cotton aphid *Aphis gossypii* on okra crop** - Wakuma Bayissa[1], Ekesi Sunday[2], Samira Mohamed[2], Nguya Maniania[2]; [1]Jimma University College of Agriculture and Veterinary Medicine, Jimma, Ethiopia; [2]ICIPE, Nairobi, Kenya

15:30 **237** **Aprehend™ for bed bug control – the biological advantag**e - Nina Jenkins, Giovani Bellicanta, Alexis Barbarin, Matthew Thomas; Penn State Department of Entomology, United States

16:00 - 16:30 - *Bourgueil*

Student Business Meeting

18:00 - 01:00

BANQUET
Grange de Meslay

18:00	Bus departure	*Outside Vinci*
until 1am	*Entertainment by Lebel Orchestre*	

POSTERS

Poster Session – Wednesday 10:30-13:00

10:30-13:00 *Agnès Sorel*

Bacteria Division

BA-1 **A novel protein active from a Pseudomonas strain with unique mode of action against western corn rootworm, *Diaborica virgifera virgifera* (LeConte)** - Nuria Jiménez-Juárez, DuPont Pioneer, USA

BA-2 **Alkaline phosphatases are involved in the response of mosquito larvae to intoxication with Bti Cry toxins** - Guillaume TETREAU, CNRS-IFREMER: UMR5244, Université de Perpignan Université de Montpellier, OMS/WHO, Perpignan, France

BA-3-STU **Aquaporins contribute to water influx into Sf9 cells intoxicated by *Bacillus thuringiensis* Cry toxin** - Haruka Endo, Tokyo University of Agriculture and Technology, Research Fellow of Japan Society for the Promotion of Science, Japan

BA-4 Association of cry genes from Bacillus thurigiensis with mortality in Spodoptera frugiperda - Newton Carneiro, Embrapa Maize and Sorghum

BA-5-STU Biological Control of *Hypsipyla Grandella* Zeller (Lepidoptera: Pyralidae) With The Systemic Use Of *Bacillus Thuringiensis* Berliner On Mahogany Seedlings (*Swietenia macrophylla* King) - Marcelo Castro, Universidade de Brasília, Brazil

BA-6 **Biomphalysin, a bacterial β-PFT family in the schistosomiasis vector snail, *Biomphalaria glabrata*** - Silvain PINAUD, CNRS-IFREMER: UMR5244, Université de Perpignan Université de Montpellier, OMS/WHO, Perpignan, France

BA-7 **Cadherins are Cry5B Toxin Receptor in Caenorhabditis elegans and Play a Sequential Role with the Glycolipid Receptor** - Ming Sun, Huazhong Agricultural University, China

BA-8 **Characteristics of an entomopathogenic bacterium, *Xenorhabdus hominickii* ANU1 and its pathogenicity against two lepidopteran pests** - Youngjin Park, Department of Bioresource Sciences, Andong National University, South Korea

BA-9 **Characterization of a Wolbachia strain native from Argentina for potential application as mosquito control agent** - Corina Berón, Instituto de Investigaciones en Biodiversidad y Biotecnología, Fundación para Investigaciones Biológicas Aplicadas, Argentina

BA-10-STU Cancelled

BA-11-STU **Dam overexpression impacts motility and virulence of the entomopathogenic bacteria, Photorhabdus luminescens TT01** - Amaury Payelleville, DGIMI, Montpellier, France

BA-12-STU **Detection and characterization of Parasporin proteins in *Bacillus thuringiensis* -** Elias, Ferreira Sabia Jr, Embrapa, Brasil

BA-13-STU **Dual action of *Bacillus thuringiensis* in the vegetative development of cotton (*Gossypium hirsutum* L.) and the control of *Spodoptera frugiperda* (Lepidoptera: Noctuidae)** - Flávia Costa, Universidade de Brasília, Brazil

BA-14 **Evidences for cross-order activity of binary Vip proteins** - Baltasar Escriche, Estructura de Recerca Interdisciplinar en Biotecnologia i Biomedicina, Departmento de Genética, Universitat de València, Spain

BA-15-STU **Evolution of *Photorhabdus* Virulence Cassettes** - Joseph Healey, Warwick University Medical School, UK

BA-16 **Genomic and phenotypic analysis of Bacillus thuringiensis cry- exposed for in vivo experimental evolution in *Galleria mellonella*** - Christina NIelsen-Leroux, Micalis, France

BA-17-STU **Mechanisms involved in the acquistion of host iron ferritin by the opportunistic insect pathogen *Bacillus cereus*** - Laurent Consentino, MICALIS équipe Génétique Microbienne et Environnement, France

BA-18 **Mosquitocidal activity of non-3-domain Cry type 33-kDa protein from *Bacillus thuringiensis* isolated in Japan** - So Takebe, Faculty of Biology-Oriented Science and Technology, Kindai University, Japan

BA-19 **Multifaceted aspects of insect pathogenic and commensal bacteria in insect based food and feed** – Christina Nielsen Leroux, INRA Micalis France

BA-20 Cancelled

BA-21 **New entomopathogenic bacterial strains from *Galleria mellonella* larvae infected with EPNs** - Luca Ruiu, Bioecopest Srl, Department of Agriculture, University of Sassari, Italy

BA-22 **Preparation and formulation optimization of a mosquitocidal sustained-release *Bacillus thuringiensis* with high UV-resistance** - Lingling Zhang, Key Laboratory of Biopesticide and Chemical Biology, Ministry of Education, Fujian Agriculture and Forestry University, China

BA-23-STU **Resistance of different *Spodoptera frugiperda* populations to Bt-maiz from the Bahía and Góias states correlates with low alkaline phosphatase expression** - Cristina Macedo, Universidade de Brasília, Brazil

BA-24 **Spent Juncao substrate can be converted into fermentable sugar with one-step method** - Yueting Xiong, Key Laboratory of Biopesticide and Chemical Biology, Ministry of Education, Fujian Agriculture and Forestry University, China

BA-25 **Structural analysis of mosquitocidal toxin sequences from a *Bacillus thuringiensis* native strain** - Corina Berón, Instituto de Investigaciones en Biodiversidad y Biotecnología, Fundación para Investigaciones Biológicas Aplicadas, Agrentina

BA-26-STU**dy of *Bacillus thuringiensis* Cry toxin binding sites in the two important soya pests *Anticarsia gemmatalis* and *Chrysodeixis includens* -** Yolanda Bel, Departmento de Genética, Universitat de València, ERI de Biotecnología y Biomedicina, Universitat de València, Spain

BA-27 **Synergism of Cry1Ac and Cry1Ie toxins and its potential for resistance management** - Kanglai He, IPPCAAS, China

BA-28 **Temperature restriction in *Photorhabdus luminescens*** - Alexia Hapeshi, Warwick University Medical School, UK

BA-29-STU **Use of *Caenorhabditis elegans* as model for selection of *Bacillus spp*. toxic strains to Meloidogyne incognita race 3** - Sandro Montalvão, Universidade de Brasília, Brazil

BA-30 **Vip3Aa laboratory selection and characterization of resistance in *Heliothis virescens* (Lepidoptera: Noctuidae)** - Juan Ferré, ERI de Biotecnología y Biomedicina, Universitat de València, Spain

10:30-13:00 *Agnès Sorel*

Diseases of Beneficial Invertebrates Division

DBI-1-STU **A New Phylogeny and eDNA Insight into Paramyxids: an Increasingly Important but Enigmatic Clade of Protistan Parasites of Marine Invertebrates** - Georgia Ward, Natural History Museum, Centre for Environment, Fisheries and Aquaculture Science, University of Exeter, UK

DBI-2 **A new simple and universal method for interactomic Studies** - Guillaume TETREAU, CNRS-IFREMER: UMR5244, Université de Perpignan Université de Montpellier, OMS/WHO, Perpignan, France

DBI-3 Cancelled

DBI-4 **Honey bee immunity: Its modulation by dietary supplements and probiotics** - Pavel Dobes, Masaryk University, Czech Republic

DBI-5-STU **Identification of *Serratia marcescens* infection in industrial rearing of *Tenebrio molitor*** - Zoé Tourrain, INRA- Micalis, France

DBI-6-STU **Identification of the honeybee parasitic mite Varroa destructor resistance using discrimination concentrations of acaricides in vitro** - Doskocil Ivo, Department of Microbiology, Nutrition and Dietetics, Czech University of Life Sciences, Czech Republic

DBI-7-STU **Occurrence of Gammaproteobacteria in honey bee gut infected by *Paenibacillus* larvae** - Zuzana Hroncova, Czech University of Life Sciences Prague, Czech Republic

DBI-8 **Ontogeny of the immune system in harlequin ladybird, *Harmonia axyridis*** - Pavel Dobes, Masaryk University, Brno, Czech Republic

DBI-9-STU **Pathogens of *Carcinus maenas* in their invasive range** - Jamie Bojko, Cefas, UK

DBI-10-STU **Ultrastructural analysis of antennal gland in American lobster experimentally infected with White Spot Syndrome Virus** - Louise-Marie Roux, Atlantic Veterinary College, University of Prince Edward Island, Canada

10:30-13:00 *Agnès Sorel*

Fungi Division

FU-1 **A new species of *Moelleriella* (Clavicipitaceae, Ascomycota) based on morphological and molecular data from China** - Xiuyan Wei, Key Laboratory of Biopesticide and Chemical Biology, Ministry of Education, College of Life Sciences, Fujian Agriculture and Forestry University, China

FU-2 **A new xanthone derivative from a new isolate of the entomopathogenic fungus *Moelleriella sp*.** - Xiangyun Zang, Key Laboratory of Biopesticide and Chemical Biology, Ministry of Education, College of Life Sciences, Fujian Agriculture and Forestry University, China

FU-3-STU Cancelled

FU-4 **Characterization of an α-amylase from the honey bee chalk brood pathogen *Ascosphaera apis*** - Lindan Yao, Key Laboratory of Biopesticide and Chemical Biology, Ministry of Education, College of Life Sciences, Fujian Agriculture and Forestry University, China

FU-5 **Characterization of the pathogenicity of commercial or precommercial Beauveria sp. strains against the melon fly *Bactrocera cucurbitae*** - Laurent Costet, CIRAD, Réunion, France

FU-6 **Does *Agriotes obscurus* avoid the fungal entomopathogen, *Metarhizium brunneum*?** - Alida Janmaat, University of the Fraser Valley, Canada

FU-7 **Effect of temperature on germination, radial growth and spore production of different isolates of *Beauveria bassiana*** - Medea Burjanadze, Agricultural University of Georgia, Georgia

FU-8-STU **Effects of *Metarhizium anisopliae* (Hypocreales: Clavicipitaceae) on the food consumption and mortality of Mexican fruit fly, *Anastrepha ludens* (Diptera: Tephritidae)** - Ricardo Toledo Hernández, El Colegio de la Frontera Sur

FU-9 **Efficient production of *Aschersonia placenta* protoplasts for transformation using optimization algorithms** - Zijian Gu, Key Laboratory of Biopesticide and Chemical Biology, Ministry of Education, College of Life Sciences, Fujian Agriculture and Forestry, China

FU-10-STU **Entomopathogenic fungi to control simultaneously both *Myzus persicae* (Green peach aphid) and plant diseases** - In Hui Kim, Department of Agricultural Biology, College of Agriculture, Life & Environment Science, Chungbuk National University, South Korea

FU-11-STU **Evaluation of entomopathogenic fungi as the dual control agents against both *Tetranychus urticae* (Two-spotted spider mite) and plant pathogens** - Dong Jun Kim, Department of Agricultural Biology, College of Agriculture, Life & Environment Science, Chungbuk National University, South Korea

FU-12 **Genetic diversity of *Metarhizium spp.* in grass, wheat, and forest habitats** - Juerg Enkerli, Institute for Sustainability Sciences Agroscope, Switzerland

FU-13 **Genetic structure of *Beauveria bassiana* in different habitats of a holm oak tree** - Maria Fernandez-Bravo, University of Córdoba, Spain

FU-14 Cancelled

FU-15 **Laboratory And Field Bioassays With *Beauveria Bassiana* And *Metharizium Anisopliae* Against Bark Beetles** - Daniela Pilarska, New Bulgarian University, Bulgarian Academy of Sciences, Bulgaria

FU-16-STU **Mealworm beetle - *Tenebrio Molitor* L as one of the best insect for isolation entomopathogenic fungi from soil** - Ketevan Koridze, Agricultural University of Georgia, Georgia

FU-17 ***Moelleriella fujianensis sp. nov*. (Clavicipitaceae, Ascomycota) from southeast China** - Lili Dong, Key Laboratory of Biopesticide and Chemical Biology, Ministry of Education, College of Life Sciences, Fujian Agriculture and Forestry University, China

FU-18 **Molecular characterization of indigenous Beauveria bassiana associated with coffee berry borers in Hawaii and assessments of their epizootic potential** - Louela Castrillo, Cornell University, USA

FU-19 **Natural occurrence of wireworms (Coleoptera: Elateridae) and entomopathogenic fungi in sunflower fields of Spain, and evaluation of their pathogenicity toward wireworms** – Enrique Quesada Moraga, Uniersity of Cordoba, Spain

FU-20 **Production of blastospores by Brazilian strains of four entomopathogenic fungi using submerged liquid culture fermentation** - Vanessa Duarte, University of São Paulo, Brazil

FU-21 **Secreted lipase as a molecular marker for *Beauveria bassiana*** - Georgy Lednev, All-Russian Institute of Plant Protection, Russia

FU-21 **Self-Defense: Insect pupal cells with antibiotic properties** - David Shapiro-Ilan, USDA-ARS, USA

FU-23 **The complete genome of Metarhizium rileyi, a key fungal pathogen of Lepidoptera** - Daniel Sosa-Gomez, Brazilian Agricultural Research Corporation, Brazilian Agriculture Research Corporation

FU-24 **Tradeoffs of immune system function with longevtiy as mediated by diet** - Parvin Shahrestani, California State University, Fullerton

FU-25-STU **Update of knowledge about *Leptolegnia chapmanii* as an agent of biological control of mosquito *Aedes aegypti*** - Claudia Lopez Lastra, Centro de estudios parasitologicos y de vectores, Argentina

FU-26 **Virulence of selected entomopathogenic fungi against the olive fruit fly and their potential for biocontrol** - Melanie Tannieres, European Biological Control Laboratory, France

10:30-13:00 *Agnès Sorel*

Microbial Control Division

MC-1-STU Cancelled

MC-2 **Aphicidal potential and virulence of *Lecanicillium* fungi from Argentina** - Romina Manfrino, Centro de Estudios Parasitológicos y de Vectores, Argentina

MC-3 **Biofilm fermentation for the production of insect pathogenic fungi** - Thomas Bawin, Functional and Evolutionary Entomology, Gembloux Agro-Bio Tech, University of Liege, Belgium

MC-4 **Biological control of *Tuta absoluta* (Meyrick) (Lep: Gelechiidae) by the use of entomopathogenic fungi** - Fatma Acheuk, Univsersité de Boumerdes, Algeria

MC-5 **BioZec - Development of a biological tick control agent based on an innovative attract-and-kill strategy** - Anant Patel, Bielefeld University of Applied Sciences, Germany

MC-6-STU **Detection of natural antagonists in *Drosophila suzukii* – a chance for biological control of the invasive insect pest?** - Sarah Biganski, Julius Kühn-Institut, , Institute for Biological Control, Germany

MC-7 **Can Endophytic Beauveria or Metarhizium Control the Wheat Stem Sawfly?**- Stefan Jaronski, USDA-ARS, USA

MC-8-STU **Development of nanoencapsulated, particle-based bait prepared with bioactive and biocompatible entomopathogenic agents for the control of leaf-cutting ants (*Atta* and *Acromyrmex* sp.) and its cultivated fungi (*Leucoagaricus gongylophorus*) as an eco-friendly alternative for sustainable agriculture**. - Esteve A. Mesén-Porras, Centro de Investigación en Biología Celular y Molecular, Costa Rica

MC-9 **Identification of new *Bacillus thuringiensis* (Berliner) isolates as biological control agents against *Ostrinia nubilalis* (Hübner) larvae** - Isabel Matas Casado, Instituto de Agrobiotecnología CSIC-UPNA, Spain

MC-10 **Impacts of entomopathogenic fungi on biology and behaviour of the invasive Brown Marmorated Stink Bug (Hemiptera, Pentatomidae)** - Thomas Bawin, Functional and Evolutionary Entomology, Gembloux Agro-Bio Tech, University of Liege, Belgium

MC-11 **Interaction of commercial products based on *Bacillus thuringiensis* and *Cotesia flavipes* (Hymenoptera: Braconidae) to the control of *Diatraea saccharalis* (Lepidoptera: Crambidae)** - Caroline De Bortoli, Sao Paulo State University, Brazil

MC-12 **Isolation and characterization of *Bacillus thuringiensis* strain from *Podisus nigrispinus* (Hemiptera: Pentatomidae)** - Caroline De Bortoli, Sao Paulo State University, Brazil

MC-13 **Isolation and identification of a *Serratia marcescens* protease with toxic activity against larvae of *Phyllophaga blanchardi* (Coleoptera: Scarabaeidae)** - María Eugenia Nuñez-Valdez, Instituto de Investigación en Ciencias Básicas y Aplicadas, Universidad Autónoma del Estado de Morelos, Mexico

MC-14-STU **Molecular identification and biological activity of African isolates of PhopGV on *Tuta absoluta* larvae** - Saoussen Ben Tiba, Regional Center for Agriculture, Chott Meriem, Tunisia

MC-15 **The use of auto-contamination-dissemination strategy for emerald ash borer (*Agrilus planipennis*) population management and development of a molecular tool for tracking the released native *Beauveria bassiana* (Bb) isolate** - George Kyei-Poku, Great Lakes Forestry Centre, Sault Ste Marie, Canada

MC-16 **Using an Antarctic fungus as a wintertime biopesticide** - Steven Edgington, CABI, UK

MC-17-STU **Virulence of vegetative insecticidal proteins Vip3Aa60 and Vip3Ad5 of *Bacillus thuringiensis* against *Spodoptera exigua* (Lepidoptera)**. - Zhizhen Pan, Key Laboratory of Biopesticide and Chemical Biology, Fujian Agriculture and Forestry University, Ministry of Education, Fuzhou, China

MC-139 **Characterization of a Colombian entomopathogenic virus isolated from the sugarcane borer *Diatraea* spp. (Crambidae)** - Gloria Barrera[1], Carolina Ruiz[1], Juliana Gómez[1], Paula Esquinas[2], Laura Villamizar[1]; [1]Corporación Colombiana de Investigación Agropecuaria (Corpoica), Cundinamarca, Colombia; [2]Universidad Nacional de Colombia, Colombia

10:30-13:00 *Agnès Sorel*

Microsporidia Division

MI-1 **Experimental infection of *Loxostege sticticalis* (Lepidoptera: Pyraloidea) with microsporidia** - Julia Malysh, All-Russian Institute of Plant Protection, Russia

MI-2 **Horizontal transmission of the microsporidium *Nosema adaliae*, from the two-spotted lady beetle, *Adalia bipunctata*, to the green lacewing, *Chrysoperla carnea*** - Susan Bjornson, Department of Biology, Saint Mary's University, Canada

MI-3-STU **The anti-Nosema active substances from entomopathogenic fungal cultures** - See Nae Lee, Department of Agricultural Biology, College of Agriculture, Life & Environment Science, Chungbuk National University, South Korea

MI-4-STU **The effects of RNAi to microsporidian parasites *Nosema ceranae* in the honeybee** - Won Seok Gwak, Department of Agricultural Biology, College of Agriculture, Life & Environment Science, Chungbuk National University, South Korea

MI-5 **The Proboscis Extension Response as a behavioral tool for assessing the vectorial competence along the life cycle of *R. prolixus* (Hemiptera: Reduviidae)** - Nadine FRESQUET, Institut de Recherche sur la Biologie de l'Insecte, UMR CNRS 7261, France

10:30-13:00 *Agnès Sorel*

Nematode Division

NE-1-STU **An entomopathogenic nematode extends its niche by associating with different symbionts** – Mohamed Asayiah, Maynooth University, Ireland

NE-2 **Biological control of large pine weevil with entomopathogenic nematodes: on the way to large scale application** - Julien Chuche, Maynooth University, Ireland

NE-3 **Concurrent Transcriptional Profiling Of Dirofilaria Immitis And Its Wolbachia Endosymbiont Throughout The Nematode Life Cycle Reveals Coordinated Gene Expression** - Barton Slatko, New England Biolabs, USA

NE-4 Cancelled

NE-5 **FIM Track: a novel method for tracking of *Drosophila* larval behavior in response to entomopathogenic nematodes** - Martin Kunc, Institute of Experimental Biology, Faculty of Science, Masaryk University, Czeck Republic

NE-6 **Heme Acquisition in the Human Parasitic Filarial Nematode, *Brugia malayi*** - Barton Slatko, New England Biolabs, USA

NE-7 **Historical review of entomopathogenic nematode research in Korea** - Dong Woon Lee, Kyungpook National University

NE-8 ***In vivo* efficacy of *Heterorhabditis bacteriophora* on *Cephalcia tannourinensis*, pest of the Cedar natural forests of Lebanon** - Martine Rehayem, Université de Montpellier, France

NE-9 **Molecular Diagnostics of Human Nematode and Protozoan Gastrointestinal Parasites in Rural Argentina, with Impact on Intestinal Microbiota** - Barton Slatko, New England Biolabs, USA

NE-10 **Optimizing the efficacy of entomopathogenic nematodes for the control of annual bluegrass weevil, *Listronotus maculicollis*, larvae** - Albrecht Koppenhöfer, Department of Entomology, Rutgers University, USA

NE-11-STU **Pheromone mediated attraction and maturation in Steinernema adults** – Cathryn Hartley, Maynooth University, Ireland

NE-12 **RNAi-mediated gene silencing of candidate drug targets in the filarial nematode *Brugia malayi*** - Silvia Libro, New England Biolabs, USA

NE-13 **Selective DNA Enrichment, High Quality Library Construction and Quantitation For Robust NextGeneration Sequencing** - Barton Slatko, New England Biolabs, USA

NE-14-STU **Soil as a Habitation of Biological Control Agents for Pest Management** - Mariam Chubinishvili, Agricultural University of Georgia, Georgia

NE-15 Cancelled

NE-16 **Survival time and infectivity of entomopathogenic nematodes with or without pre-conditioning formulated in alginate beads** - Jaime Ruiz-Vega, Instituto Politecnico National, CIIDIR-Oaxaca, Mexico

NE-17 **Susceptibility of Mealy Plum Aphid, Hyalopterus Pruni (Homoptera:Aphididae), to entomopathogenic nematodes Steinernema carpocapsae and Steinernema feltiae (Rhabditida; Steinernematidae) under laboratory conditions** - Nona Mikaia, Sokumi State University, Georgia

NE-18 **Temperature effects on Korean isolated entomopathogenic nematode, Steinernema kraussei** - Dong Woon Lee, Kyungpook National University, South Korea

10:30-13:00 *Agnès Sorel*

Virus Division

VI-1 **A new virus from *Cotesia* parasitoid wasps fills a gap in the arthropod large dsDNA virus phylogeny** - Annie Bézier, Institut de Recherche sur la Biologie de l'Insecte, UMR CNRS 7261, France

VI-2 **A Shannon entropy-based method to predict the localization of transmembrane proteins (BV or ODV envelopes) in the Baculoviridae family** - Mariano Belaich, Universidad Nacional de Quilmes, Argentina

VI-3 **Adaptation of a Colombian Spodoptera frugiperda nucleopolyhedrovirus isolate to alternative host *Heliothis virescens*** - Gloria Barrera, Corporación Colombiana de Investigación Agropecuaria, Columbia

VI-4 **Analysis of protein expression from the baculovirus AcMNPV in the cell line BTI-TN-5B1-4 at different post-infection times** - Cristina Del Rincón-Castro, University of Guanajuato, Mexico

VI-5 **Baculovirus infection induces disassembly of nuclear lamina** - Meijin Yuan, State Key Laboratory of Biocontrol, Sun Yat-sen University, China

VI-6-STU **Baculovirus ODV occluded by polyhedra in different insect cell lines** - Riyadh Abdulsahib Alakeely, Oxford Brookes University, UK

VI-7-STU **Biochemical characterisation of the baculovirus per os infectivity complex** - Bob Boogaard, Laboratory of Virology, Wageningen University, Netherlands

VI-8 **Characterization of VP91 of Helicoverpa armigera nucleopolyhedrovirus** - Fengqiao Zhou, State Key Laboratory of Virology and Joint Laboratory of Invertebrate Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, China

VI-9 **Co-infection by SeIV iflavirus and Spodoptera exigua multiple nucleopolyhedrovirus (SeMNPV): effects on occlusion body structure and conformation** - Rosa Murillo, Instituto de Agrobiotecnologia CSIC-Gobierno de Navarra, Departamento de Produccion Agraria, Universidad Publica de Navarra, Spain

VI-10-STU **Comparison of genome replication rates of fast-killing versus slow-killing SfAV isolates** - Hiroki Ishii, Tokyo University of Agriculture and Technology, Japan

VI-11 **Development of a highly efficient recombinant system for Anticarsia gemmatalis multiple nucleopolyhedrovirus (AgMNPV) and findings about baculoviral replication in absence of the essential gene orf1629** - M. Leticia Ferrelli, Instituto de Biotecnología y Biología Molecular, Argentina

VI-12 **Does AcMNPV loose its generalist potential when adapting to a specific host? –** Yannis Moreau, Institut de Recherche sur la Biologie de l'Insecte, UMR CNRS 7261, France

VI-13 **Earthworm mediated dispersal of baculovirus occlusion bodies in soil** - Trevor Williams, Instituto de Ecología AC, Mexico

VI-14-STU **Genetic and biological characterisation of a novel alphabaculovirus for the microbial control of *Cryptophlebia peltastica*** - Tamryn Marsberg, Department of Zoology and Entomology, South Africa

VI-15 **Genomic Analysis of Four Plutella xylostella Granulovirus Isolates** - Robert Spence, Queensland University of Technolgy, Australia

VI-16 **Impact of single and multiple morphotypes on genome-wide selection in baculovirus** - Fernando Melo, Universidade de Brasília, Brazil

VI-17-STU **Improving Baculovirus Surface Display System** - Mine Aksular, Oxford Brookes University, Oxford Expression Technologies, Pirbright Institute, UK

VI-18 **Insect immune system to determine baculoviruses host specificity –** Yu Wei Chen, National Taiwan University, Taiwan

VI-19-STU **Insecticidal activity of Phthorimaea operculella granulovirus isolates from Southern Europe on *Tuta absoluta*** - Eduardo Aguirre, Departamento de Producción Agraria, Universidad Pública de Navarra, Instituto de Agrobiotecnología, Spain

VI-20-STU **Insecticidal evaluation of a recombinant Trichoplusia ni granulovirus (TnGV) generated by biolistics** - Juventino López-Tlacomulco, Centro de Investigacion y de Estudios Avanzados del Instituto Politécnico Nacional, Mexico

VI-21-STU **Is ORF1629 essential or not for the replication of baculovirus?** - See Nae Lee, Department of Agricultural Biology, College of Agriculture, Life & Environment Science, Chungbuk National University, South Korea

VI-22-STU **Light at a fixed time period after infection is needed for Spodoptera exigua MNPV-induced tree-top disease** - Yue Han, Wageningen University, Netherlands

VI-23-STU **Morphological properties of the occlusion body of Adoxophyes orana granulovirus** - Keiko Tsuruta, Tokyo University of Agriculture and Technology, Tokyo, Japan

VI-24 **New Polydnavirus genomes of *Microgaster* wasps** - Philippe Gayral, Institut de Recherche sur la Biologie de l'Insecte, UMR CNRS 7261, France

VI-25-ST **Novel Cydia pomonella granulovirus isolates break virus resistance in codling moth** - Jiangbin Fan, Insitute for Biological Control, Julius Kühn Institute, Darmstadt, Germany, Key Laboratory of Plant Protection Resources and Pest Management of Ministry of Education, Northwest A&F University, China

VI-26 **Nuclear translocation signal of AcMNPV ME53 influences overall virus production while the Zn finger is important for virus production early in infection** - Peter Krell, University of Guelph, Canada

VI-27-STU **Overview of bee viruses in wild hymenoptera** - Diane Bigot, Institut de Recherche sur la Biologie de l'Insecte, UMR CNRS 7261, France

VI-28 **Polydnavirus-Encoded MicroRNA exerts different effects on the immune responses in *Spodoptera litura* (Fabricius) and *Snellenius manilae* (Ashmead)** - Yueh-Lung Wu, Department of Entomology, National Taiwan University, Taiwan

VI-29 **Role of lef-5 from SeMNPV in the stability of baculovirus in cell culture** - Salvador Herrero, Estructura de Recerca Interdisciplinar en Biotecnologia i Biomedicina (, Universitat de València, Spain

VI-30-STU **Spodoptera exigua iflavirus co-inoculation alters the insecticidal properties of Spodoptera exigua multiple nucleopolyhedrovirus (SeMNPV) occlusion bodies** - Arkaitz Carballo, Departamento de Produccion Agraria, Universidad Publica de Navarra, Instituto de Agrobiotecnologia CSIC-Gobierno de Navarra, Spain

VI-31 **Spodoptera frugiperda granulovirus: genomic organization of an Argentinean isolate -** María Leticia Ferrelli, Instituto de Biotecnología y Biología Molecular, Universidad Nacional de La Plata-CONICET, Argentina

VI-32 **ssRNA viruses discovery in the fresh water snail Biomphalaria sp, the intermediate host of intestinal Schistosomiasis** - Richard Galinier, CNRS-IFREMER: UMR5244, Université de Perpignan Université de Montpellier, OMS/WHO, Perpignan, France

VI-33-STU **Studies on the role of putative replication origins from the nucleopolyhedrovirus of *Anticarsia gemmatalis* using an in vitro coinfection method** - Mariano Belaich, Universidad Nacional de Quilmes, Argentina

VI-34 **Survey for Oryctes rhinoceros nudivirus (OrNV) in a Hawaiian coconut rhinoceros beetle (*Oryctes rhinoceros*) population and genetic diversity of Pacific isolates of OrNV** - Shizu Watanabe, University of Hawaii, USA

VI-35 **The Autographa californica multiple nucleopolyhedrovirus ac110 Gene Encodes a New Per Os Infectivity Factor** - Wenbi Wu, Sun Yat-sen University, China

VI-36 Cancelled

VI-37 Cancelled

VI-38 **The profiling of six miRNAs encoded by AcMNPV** - Jinwen Wang, Sun Yat-Sen University, China

VI-39 **Virome composition of *Apis mellifera* colonies infested with Varroa destructor** - Nor Chejanovsky, Entomology Department, Inst Plant Protection, Agricultural Research Organization, Israel

VI-40 **Virus discovery and applications for the management of snail-vectored human disease** - Bryony Bonning, Iowa State University, USA

ABSTRACTS 2016

Important Notes

Attendants shall not take pictures from projections during presentations

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STU indicates STUDENT presentation
000 indicates the number of ORAL presentation
BA – 00 indicates abstract number for POSTER presentation

SUNDAY – 24 July

BACTERIA WORKSHOP

Sunday, 14:00-17:00 - ***Chinon***

The future of the Bt nomenclature

The future of the Bt nomenclature

Colin Berry
School of Biosciences, Cardiff, United Kingdom

The current nomenclature for Bt toxins has been of great value to the field in providing a structured system for the allocation and assignment of toxin names. However, advanced sequencing technologies are producing new data at a rate that is overwhelming the manually curated system and a solution to this problem must be found to allow continuity of a unified nomenclature system. It is proposed to establish a revised nomenclature system that will encompass a wider range of microbial, invertebrate-active toxins. The system will need to maintain the essential features of the current nomenclature eg unique identifiers for each toxin sequence and providing indications of how different toxins are related. The aim will be to have layers of analysis beneath the basic nomenclature, which can achieve things such as domain-level comparisons and ultimately make associations between structure and function (eg activity against a particular target). Most steps will be automated in order to allow processing of large numbers of new sequences. The concept of this new nomenclature is supported by many academic and industrial partners in the field. This workshop will discuss the issue of nomenclature and collect ideas from users in order that we can devise a system that is suitable for their needs.

MONDAY – 25 July

PLENARY SYMPOSIUM

Monday, 10:30-12:30 - ***Descartes***

Insect for Food and Feed - *Christina Nielsen Leroux*

SYMPOSIUM. Monday, 10:30 **1**

Opportunities and Constraints of farming Insects for food and feed: a global review

Paul Vantomme
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Trends towards 2050 predict a steady population increase to 9 billion people. Particularly the demand for animal proteins is exploding. "New" plant and animal species as sources of proteins are being investigated such as: algae (*Spirula*), Moringa, medusae and jelly fish or even laboratory-made artificial meat. However, farming "insects" appear the most promising. Insects are part of the traditional diets already of approximately 2 billion people worldwide. Insects can contribute to food security given their high nutritional value, low emissions of greenhouse gases (GHG), low requirements for land and water, and the high efficiency at which they can convert feed into food. The majority of insects consumed in developing countries today are harvested in nature. In western countries, the disgust factor to consider insects as food, combined currently with their limited availability on the market and a lack of regulations governing insects as food and feed are major barriers for their further expansion. The overall contribution of edible insects to livelihoods is difficult to estimate by lack of reliable statistics. However, the biggest opportunity may well lay in the production of insect biomass as feedstock for animals as it can be combined with the bioprocessing of organic waste. Considering the immense quantities of insect biomass needed to supplement current protein-rich feed ingredients, automated mass rearing facilities that produce stable, reliable and safe products need to be developed. For this to occur significant technological innovations, changes in consumer food preferences, insect-encompassing food and feed legislation, and progress towards more sustainable food production systems are needed.

SYMPOSIUM. Monday, 11:00, **2**

Industrialization of Insect Farming: New challenges to prevent pathogenic hazards

Thomas Lefebvre
YNSECT, Genopole, Evry, France

In recent years, insect farming has experienced a genuine worldwide expansion through diverse applications in biological control, chemical industries, animal feed or human food. Nowadays, the activity is developing at industrial scale projecting to produce more than dozens kilotons of insects per year. This important scale-up raises several major challenges for technological innovations but also for health and safety issues. Actually, the most damageable risk to be considered for an insect farm should be the disease outbreak. However, the knowledge of pathogens and parasites of insects is still very limited, and new pathologies emergence could also appear with the rise of insect mass production. In this domain, only millenary practices of beekeeping or sericulture benefit from extensive knowledge on prophylaxis, pathogens detection and therapeutic methods. Finally, the success of this emergent agroindustrial sector based on insect farming depends a lot on effective infectious risk management. The organization of insect producers for feed and food (IPIFF), and especially Ynsect, are concerned about this issue and are developing research programs, rearing units monitoring and quality procedures to prevent health hazards. In short, the purpose of the speech is to present the insect farming sector through its industrialization and to discuss about the measures taken to manage infectious risks.

SYMPOSIUM. Monday, 11:30, 3

Managing insect viruses in insect factories for food and feed:
Successful management of an insect virus, Glossina pallidipes salivary gland hypertrophy virus, from an insect factory

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

Recently there is an increasing need for the production of insect in large scale for several purposes, which include: the production of benefit insect, as host for the production of biological control agents, for the sterile insect technique and as food and feed. The maintenance of large scale insect mass rearing factories faces many challenges, but the control of insect viral diseases represent the main challenge and determine the sustainability of the insect factories. As a model for successful management strategy of viral infection in insect factory was recently demonstrated in tsetse fly factory in Ethiopia. Tsetse flies are naturally infected by *Glossina* hytrosavirus, a large dsDNA virus pathogenic to the *Glossina* spp. The virus infection is characterized by salivary gland hypertrophy syndrome (SGH), leading to reproductive dysfunction of infected flies and colony collapse. 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: identifying the virus transmission, understanding tsetse biology and rearing system, reducing the virus replication and infection using available antiviral drugs and interrupting the virus transmission by optimizing and modifying tsetse feeding system to a clean feeding system. This management strategy 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 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.

SYMPOSIUM. Monday, 12:00, 4

Pathogenic aspects in insects produced for feed and food

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Over the last few years there has been an immense increase in the production of insects for feed and food. Such invertebrate 'mini livestock' will meet the same type of challenges as other livestock production in order to maintain a healthy stock. A major potential threat to farmed insects is the occurrence of insect diseases, which need to be prevented or managed. There will presumably be an increased demand for versatile insect pathologists, diagnostic manuals and educational workshops. Also, since the farmed insects are to be used as food or feed, there are potentially also safety problems, if microorganisms in the insect production systems pose a health hazard to the consumers, vertebrate livestock or humans. There can even be opportunistic microorganisms, which may infect insects in dense cultures and cause production losses, and at the same time are unwanted from a food and feed safety perspective. I will give an overview of state of the art on the subject, with examples from some of the most important farmed insects: House cricket *Acheta domesticus*, Black soldier fly *Hermetia illucens*, mealworm *Tenebrio molitor* and others. As part of our efforts, we made a survey (started 2014 and with follow ups) asking insect producers, which disease problems they have noticed and which action plans were initiated.

DISEASES OF BENEFICIAL INVERTEBRATES SYMPOSIUM — Monday, 14:00-16:00 - ***Courteline***

Mollusc diseases - *Grant Stentiford*

SYMPOSIUM. Monday, 14:00, 5

New perspective on the microcell parasites

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Microcell parasites include small intracellular protistan parasites of the two genera *Bonamia* and *Mikrocytos*. These tiny unicellular parasites have been associated with mortality of oysters in different parts of the world. This impact on the oyster populations has led to economic losses and has motivated the development of research works to resolve their phylogenetic position, their life cycle and better understand their interactions with their hosts. Our understanding of these microcell parasites has expanded in recent years notably thanks to the development of new specific detection tools such as PCR, *in situ* hybridization and the use of New Generation Sequencing approach. Recent works have confirmed the phylogenetic placement of *Mikrocytos* within Rhizaria and as a sister taxon to Haplosporidia which includes the genus *Bonamia*. New species have been characterized in oysters but also in clams and 3 species are currently acknowledged in each genus *Bonamia* and *Mikrocytos.* However, the lack of molecular data currently hampers the characterization of some potentially additional species. Improving our understanding of the diversity and distribution of these parasites is still needed notably to assess detection methods for EU regulated pathogens such as *M. mackini*, *B. ostreae* and *B. exitiosa.* While infection dynamics inside bivalves is pretty well understood, the forms and distribution outside oysters remain unknown. The evolution and ecology of these parasites is a ripe area for future research.

SYMPOSIUM. Monday, 14:30, 6

A new phylogeny and eDNA insight into paramyxids:
an increasingly important but enigmatic clade of protistan parasites of marine invertebrates

Georgia Ward[1], Martyn Bennett[2,3], Kelly Bateman[2], Grant Stentiford[2], Rose Kerr[2], Stephen Feist[2],
Suzanne Williams[1] Cedric Berney[1], David Bass[1,2†]
1 Natural History Museum, London, United Kingdom; 2 CEFAS, Weymouth, United Kingdom; 3 School of Biosciences, Univ. of Exeter, United Kingdom
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Paramyxida is an order of rhizarian protists that parasitise marine molluscs, annelids, and crustaceans. They include notifiable pathogens (*Marteilia* spp) of bivalves and other taxa of economic significance for shellfish production. The diversity of paramyxids is poorly known and their phylogenetic position is unclear due to their extremely divergent 18S rDNA sequences. However, novel paramyxean lineages are increasingly being detected in a wide range of invertebrate hosts. We review the diversity, host affiliations, and geographical ranges of all known paramyxids, present a comprehensive phylogeny of the order, and clarify its taxonomy. Our phylogenetic analyses confirm the separate status of four genera: *Paramarteilia*, *Marteilioides*, *Paramyxa*, and *Marteilia*. Further, as including *M. granula* in *Marteilia* would make the genus paraphyletic; we suggest transferring this species to a new genus, *Eomarteilia*. We present sequence data for *Paramyxa nephtys* comb. n., a parasite of polychaete worms, providing morphological data for a clade of otherwise environmental sequences, sister to *Paramarteilia,* and a new *Paramyxa* sp. infecting oysters and mussels in the UK. We provide histological and EM data for *Paramarteilia orchestiae*, the type species of that genus originally described from the amphipod *Orchestia. Paramarteilia* also infects crab species and is implicated in recent severe mortality events in commercial crab fisheries. We present the first results of a paramyxid-specific environmental DNA (eDNA) survey of environmental and organismally-derived samples, revealing new lineages and showing that paramyxids are associated with a wider range of hosts and habitat types than previously known.

SYMPOSIUM. Monday, 15:00, 7

Viral diseases affecting marine bivalves

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The study of shellfish diseases is a relatively young science and the discovery of viruses in marine molluscs is a fairly recent event. Viruses interpreted as members of various families (*Papovaviridae*, *Togaviridae*, *Retroviridae*, *Reoviridae*, *Birnaviridae* or *Picornaviridae*) have been described in marine bivalves. There is currently a lack of information concerning the occurrence of mollusc viruses world-wide and the basic method for identification and examination of suspect samples remains predominantly histopathology. This technique enables the identification of cellular changes associated with infection but does not provide conclusive identification of mollusc viruses unless completed by other methods such as transmission electron microscopy. Few studies have involved molecular identification and/or experimental *in vivo* trials confirming the affiliation and/or the pathogenicity of these agents. Viral pathogens are often highly infectious and easily transmissible, and are commonly associated with mass mortalities. Infections by irido-like viruses were associated with massive mortality outbreaks of *Crassostrea angulate*, herpesviruses have also been associated with disease outbreaks involving substantial mortalities in different bivalve mollusc species. Mass mortality outbreaks in association with the detection of a herpes-like virus were reported among larvae of hatchery-reared Pacific oyster for the first time in France during summer 1991. This virus based on complete genome sequencing has been identified as *Ostreid herpesvirus type 1* (OsHV-1), the presentation is focused on the present knowledge about virus diversity and viral infection process related to the experession of viral and host genes.

SYMPOSIUM. Monday, 15:30, 8

Breeding for disease resistance – Development of a *Crassostrea gigas* SNP array

Tim Bean†1, Alejandro Gutierrez[2,3], Richard Paley[1], Chantelle Hooper[1], Matthew Sanders[1], Craig Stenton[1],
Karim Gharbi[3], Ross Houston[2,3]
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Oyster herpes virus has been associated with sporadic mass mortality events farmed Pacific oysters (*Crassostrea gigas*) for over 30 years. The emergence of a new variant (denoted OsHV-1 uVar) caused losses within the *C. gigas* aquaculture industry worldwide. During these initial outbreaks mortalities in excess of 80% were regularly observed. Shifts in susceptibility of breeding populations have demonstrated that some oysters carry an inherent level of survivability when challenged by the pathogen, and that this "resistance" is heritable. In this study we are attempting to characterise this resistance using a newly-developed high density single nucleotide polymorphism array (SNP chip). To develop the array, approximately ~12 million candidate *C. gigas* SNPs were identified by whole genome sequencing of eight pools of genomic DNA from *C. gigas* oysters sampled from hatcheries and farm locations in the UK and France (number of divergently selected oyster lines from Ifremer, France). In addition, to broaden the utility of this tool, we added a selection of candidate SNPs identified by Restriction-site Associated DNA (RAD) sequencing of pooled samples from 11 populations of European flat oyster (*O. edulis*) from diverse locations in Europe (and 1 from USA). SNP filtering was based on criteria including minor allele frequency and read coverage across populations, requirement for monomorphic flanking regions, even distribution across the genome (where possible), and Affymetrix prediction of SNP conversion score. The first application of the array will be to map areas of the genome affecting resistance to Oyster Herpes Virus based on a laboratory challenge experiment on a large population of juvenile *C. gigas* from a commercial hatchery stock.

Ménage à trois: Three way interactions between plants, arthropods and microbes that benefit the plants

Richard Meadow & Maria Pozo

Contributed paper. Monday, 14:00, **9**

A fungal endophyte helps plants to tolerate root herbivory through changes in gibberellin and jasmonate signaling

Marco Cosme[1], Jing Lu[2], Matthias Erb[3], Michael Stout[4], PhilippFranken[5], Susanne Wurst[6]

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Plant–microbe mutualisms can improve plant defense, but the impact of root endophytes on below-ground herbivore interactions remains unknown. We investigated the effects of the root endophyte *Piriformospora indica* on interactions between rice (*Oryza sativa*) plants and its root herbivore rice water weevil (RWW; *Lissorhoptrus oryzophilus*), and how plant jasmonic acid (JA) and GA regulate this tripartite interaction. Glasshouse experiments with wild-type rice and *coi1-18* and *Eui1-OX* mutants combined with nutrient, jasmonate and gene expression analyses were used to test: whether RWW adult herbivory above ground influences subsequent damage caused by larval herbivory below ground; whether *P. indica* protects plants against RWW; and whether GA and JA signaling mediate these interactions. The endophyte induced plant tolerance to root herbivory. RWW adults and larvae acted synergistically via JA signaling to reduce root growth, while endophyte-elicited GA biosynthesis suppressed the herbivore-induced JA in roots and recovered plant growth. Our study shows for the first time the impact of a root endophyte on plant defense against below-ground herbivores, adds to growing evidence that induced tolerance may be an important root defense, and implicates GA as a signal component of inducible plant tolerance against biotic stress.

Contributed paper. Monday, 14:15, **10**

Uncovering the effects of cover crops and soil characteristics on *Metarhizium*-plant-insect interactions in an organic cropping system

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The use of cover crops is promoted for their benefits to soil conservation and fertility. However, the impacts of this conservation tool on soil organisms, such insect pathogens, are not well known. We will report on *Metarhizium*-insect-plant interactions in soil from a field experiment in organic maize and soybean. Treatments included a bare fallow check; 6 winter cover crop species grown in monoculture; and five winter cover crop treatments of various levels of species richness. Prevalence of *Metarhizium* was greatest in maize and soybean that followed a 6-species and 7-species winter cover crop mixture, respectively. Soil moisture, sulfur concentration and pH were positively associated with *Metarhizium* in maize; whereas zinc, sulfur and calcium concentrations were positively associated with *Metarhizium* in soybean. Preliminary sequencing of six isolates indicated at least four taxa: *M. anisopliae*, *M. majus, M. guizhouense,* and *M. robertsii.* In greenhouse assays, all isolates showed endophytic activity in roots, stems, and leaves in V4 maize grown from seed exposed to conidia. In detached leaf feeding assays, the relative growth rate of 2nd instar black cutworm, *Agrotis ipsilon*, feeding on maize infected with *M. guizhouense* and *M. robertsii* was significantly decreased by 18.8 and 20.5%, respectively, compared to the control, suggesting suppression of this pest by some of the identified isolates. In a preliminary test with *M. majus*, the expression of two genes related to plant stress, *TIP1* (Tonoplast Intrinsic Protein) and *MPI* (Maize Protease Inhibitor), increased, while *MYB* (a family of transcription factors) and *RIP2* (Ribosome Inactivating Protein 2), were down-regulated in *Metarhizium*-inoculated plants compared to control plants.

Contributed paper. Monday, 14:30, **11-STU**

Induced plant defense accomplished by a grass endophyte

Benjamin Fuchs[†], Jochen Krauss

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Endophytic fungi in cool season grass species can affect the plant surrounding ecosystem by producing a variety of chemical compounds. Alkaloids produced by the endophytic fungus *Epichlo festucae* var. *lolii* in the host grass *Lolium perenne* are known to harm herbivores and thereby enhance the grass fitness. In a multi-trophic level approach we addressed the question whether alkaloids are ingested by different herbivores and can be detected also in higher trophic levels. With UPLC-MS analysis we showed that endophyte derived alkaloids cascade up the food chain and can be detected in herbivores (aphids, locusts) and several aphid predators (lacewing larvae, hoverfly larvae, all ladybird stages). By cascading up the food chain, alkaloids harm not only the herbivores and potential pest species but also their predators with unknown ecological consequences. Plants developed different strategies to best defend against herbivores; one of them is the herbivore induced production of secondary metabolites. Induced defense is well studied for plants, but mostly unknown for plant associated microorganisms. We addressed the question whether the endophyte growth and alkaloid production is induced by different herbivores feeding on endophyte infected grass. In a controlled common garden experiment we showed that alkaloids (UPLC-MS) and fungal growth (qPCR) are induced herbivore specific. While the insect deterring alkaloid peramine was enhanced by locusts, endophyte growth and the neurotoxin lolitrem B were enhanced by simulated cattle grazing. Our results indicate a close chemical crosstalk between herbivores, plants and endophytic fungi.

Contributed paper. Monday, 14:45, **12**

Systemic grass endophytes and their importance for herbivores in Europe

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Endophytic fungi of cool season grass species are known to deter herbivores from damaging agricultural grass hosts. Phloem feeding aphids show a fitness loss when feeding on endophyte infected host grass, but knowledge on the mechanisms are fragmentary. It is also unknown whether responses of aphids differ under laboratory and field conditions and how higher trophic levels are affected by the presence of such endophytes. We showed in our experiments that alkaloids, like peramine and lolitrem b, which are produced by the *Neotyphodium lolii* – *Lolium perenne* association can be assimilated by phloem-feeding aphids. These alkaloids do not only occur in aphids, but also cascade up the food chain and might be responsible for strong negative effects in predators and parasitoids of aphids reared exclusively on *Neotyphodium lolii* infected grass. However, strong effects of the fungus – grass association on trophic cascades in laboratory experiments could rarely be found in field experiments in Central Europe. Under field conditions aphid populations often depend on top down control and the environmental context, while higher trophic levels are mainly bottom up driven by the host grass biomass. We conclude that the alkaloids produced by the endophyte – grass association are responsible for fitness loss in different trophic levels, but that this effect is relatively low under field conditions in Central Europe. We therefore started a new project on infection rates in Germany and detected an infection frequency of 13 % for *L. perenne* and 74% for *Festuca pratensis.* Peramine, lolitrem B and ergovaline were occasionally above published toxicity levels in infected *L. perenne* samples.

Contributed paper. Monday, 15:00, **13**

Plant metabolic responses to endophytic colonization by *Trichoderma* and *Epichloe* and their effect on insects

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Fungi in the genera *Trichoderma* and *Epichloe* form intricate relationships with plants by colonizing them as endophytes. Such plant-microbe interactions may change the phenotype of the plant and thus enhance resistance against herbivorous insects through antibiosis or antixenosis. In order to make better use of endophytes (including the entomopathogens *Beauveria* and *Metarhizium*) for sustainable plant protection, it is necessary to understand the mechanisms that can lead to such resistance. A limited number of studies have shown that plant inoculations with *Trichoderma* spp. can confer antibiosis against sucking herbivores. We investigated whether this is also true for leaf chewers by assessing the potential of *Trichoderma atroviride* LU 132 colonization to induce and/or prime systemic resistance (ISR) against *Plutella xylostella*. While enhanced plant growth and reduced aphid numbers were observed in *Trichoderma*-treated *Brassica napus*, no effects on *P. xylostella* larvae could be found. Measurements of phytohormones, glucosinolates and defence-related gene expression did not suggest ISR or priming effects. Further studies will need to explore whether *Trichoderma* -induced resistance is restricted to phytopathogens and sucking insects. Antibiosis in *Epichloe* -colonized grasses is well established and conferred by fungal alkaloids. We investigated the possibility of antixenosis, mediated by changes in root volatile emission. PTR-MS measurements showed that endophyte presence altered and attenuated the spectrum of volatile root compounds. The volatile blend of colonized plants attracted fewer larvae of the root feeder *Costelytra zealandica* and therefore contributed to alkaloid-mediated resistance.

Contributed paper. Monday, 15:15, **14**

Endophytic entomopathogenic *Metarhizium brunneum* against insect pests: novel integrated fermentation and formulation strategies

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Endophytic entomopathogenic fungi (EEPF) like *Metarhizium* spp. are pathogenic to many different insect orders and are able to colonize different plant species and plant parts. Plant-mediated biocontrol of insect pests with these fungi is challenging because of the low plant penetration and colonization rates, difficult application, limited shelf life, as well as an insufficient understanding on the mode of action when growing within the plants. A practical solutions, minimizing several of these problems, could be a bioprocess engineering approach that combines fine-tuned mass-production of "endophytically competent" biomass with customized formulations to obtain novel formulations that support the delivery of EEPF *into* plants for a systemic protection from insect pests. Therefore, *M. brunneum* hyphae fragments were mass-produced in 2L bioreactors and encapsulated in an innovative bead formulation. After drying beads and unformulated biomass at 30◦C viability of encapsulated hyphae fragments was 52.2 ± 37.4% compared to 0.7 ± 0.3% for unformulated biomass. Formulated dried hyphae fragments were stored for 6 month at 5◦C, 18◦C and 25◦C and maintained viability with 83.6 ± 17.4%, 55.4 ± 9.9% and 43.5 ± 23.5%, respectively. When dry beads were applied to tomato plant roots, plant colonization was positively verified via light microscopy and qPCR. Additional data of on-going experiments on endophytic virulence against *T. absoluta* and *T. vaporariorum* will be presented. To conclude, by following a bioprocess engineering approach, plant colonization by endophytic entomopathogenic fungi can be substantially improved, thus paving the way for a novel plant protection measure.

Contributed paper. Monday, 15:30, **15**

Determination of destruxin A in potato plants after foliar spray of *Metarhizium brunneum*

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Strains of entomopathogenic fungi within the genus *Metarhizium* have increasingly been developed for the control of insect pests, usually applied using an inundative approach on the crops. Risk assessment studies are a prerequisite before the fungus can be registered as a plant protection product. In this work we determined the risk posed by destruxin A (dtx A), which is the major secondary metabolite produced by the genus *Metarhizium sp*. A simple QuEChERS- based dtx A extraction has been used in four different parts of potato plants (leaves, stems, roots, and tubers) after spraying the EAMa 01/58-Su *M. brunneum* strain. Plants were evaluated 24, 48, 72, 96 and 120 h after inoculation and analyzed by high

performance liquid chromatography- tandem mass spectrometry (HPLC-MS/MS). EAMa 01/58-Su strain was able to endophytically colonize all parts of the potato plants. Stems and leaves colonization percentages (more than 60.0 and 80.0%, respectively) were higher than tubers and roots colonization percentages (both less than 10%). In fact, tubers and roots only showed colonization after 72h. Dtx A was detected in roots and tubers at 24 h (2.49 ± 1.7 and 2.0 ± 1.4 μg/L, respectively) and 96 h (2.5 ± 1.7 μg/L). These results indicate that *M. brunneum* strain may secrete the secondary metabolite since the beginning of the plant colonization, and it is rapidly and systemically transported inside the plant. However, the level of this metabolite found in plant could be tolerated for human consume by similarity with other compounds accepted by the EFSA.

CONTRIBUTED PAPERS — Monday, 14:00-16:00 - ***Vouvray***

Virus 1 - *David Theilmann & Kai Yang*

Contributed paper. Monday, 14:00, **16-STU**

Protein tyrosine phosphatase 2 from the baculovirus SeMNPV induces apoptosis in insect cells

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The family *Baculoviridae* harbours a large number of invertebrate viruses, mainly infecting caterpillars of the order Lepidoptera. The baculovirus Spodoptera exigua multiple nucleopolyhedrovirus (SeMNPV) causes several alterations in its host *S. exigua,* including physiological and behavioural changes, as well as immunological responses (apoptosis in hemocytes). It is likely that these changes in the host underlie efficient virus transmission. Here we show that the viral encoded protein tyrosine phosphatase 2 (PTP2) induces apoptosis in *Spodoptera frugiperda* (Sf) 21 cells upon transient expression. Transfection with a catalytic site mutant did not lead to apoptosis, indicating that the phosphatase activity of PTP2 was needed to induce apoptosis. We also found that the caspase level (indicator of apoptosis) was higher in cells transfected with the *ptp2* gene than in cells transfected with the *ptp2* catalytic mutant. A caspase inhibitor reduced the level of *ptp2*-induced apoptosis. PTP2 shares a functional domain with mitogen-activated protein kinase (MAPK) phosphatases (MKPs), which are important cellular proteins that regulate many cellular processes, including apoptosis and other immune responses. The phylogenetic relation between PTP2 and insect MPKs further suggests that PTP2 might have MPK activity. Overall, we hypothesize that SeMNPV PTP2 functions as a MKP and possibly induces apoptosis specifically in hemocytes to suppress immune responses. We are currently performing proteomic studies to determine which MAPK may serve as a substrate for PTP2.

Contributed paper. Monday, 14:15, **17-STU**

Characterization of AcMNPV encoded viral ubiquitin and its association with AC141 for the production of budded virus

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AcMNPV encodes a viral ubiquitin (v-ubi) which is a homolog of cellular ubiquitin (c-ubi). The precise function of v-ubi is unknown but previous studies have shown that inactivation of v-ubi results in a 5-10 fold reduction in the production of budded virus (BV). In addition, *in vitro* analyses showed that v-ubi was kinetically less favourable than c-ubi as a substrate for cellular E3-ubiquitin ligases. Proteomic analysis has also shown that v-ubi is present in BV as a free form or is conjugated to viral proteins. AcMNPV AC141 is a potential E3 ubiquitin ligase that has also been shown to be required for BV production. Due to the common impact on BV production we hypothesized that v-ubi interacts with AC141. To address this question, AcMNPV bacmids were produced that contained gene knockouts (KOs) of either *v-ubi*, *ac141* or a double KO virus (AC141+v-ubi2xKO). Sf9 cells infected with the v-ubi KO virus showed an 83% reduction in BV production. Whereas cells transfected with the AC141+v-ubi2xKO virus were restricted to single cell infection and produced no BV. The AC141+v-ubi2xKO virus was rescued with epitope tagged HA-AC141 and Myc-v-ubi. Co-immunoprecipitation analysis with HA-AC141 on total infected cell proteins identified two Myc-v-ubi bands of 35 and 45 kDa. Mass spectrometry analyses of the Myc-v-ubi bands showed that AC141 is ubiquitinated with v-ubi at K87. Western blot analysis on fractionated BV and occlusion derived virus (ODV) showed a number of nucleocapsid proteins were specifically ubiquitinated with v-ubi. In addition BV showed significantly higher levels of v-Ubi as compared to ODV. These results show that AC141 interacts with v-ubi and this interaction may be required for efficient BV production.

Contributed paper. Monday, 14:30, **18-STU**

3Dimensional ultrastructural modelling of Autographa californica multicapsid nucleopolyhedrovirus infection in insect cells to determine the role of P10 during baculovirus infection

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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 include nuclear stability, polyhedron formation and cell lysis, but distinct mechanisms to account for these roles have yet to be determined. To investigate the role of P10 during infection, Autographa californica multicapsid nucleopolyhedrovirus (AcMNPV) infected *T.ni* 368 cells were chemically fixed and resin embedded at 24, 48, 72 and 96 hours post infection (hpi) and imaged using serial block-face scanning electron microscopy (SBF-SEM) to view structures in high resolution. Using various software packages, P10 and associated structures, including electron-dense spacers (EDS) and polyhedra were modelled offering a new level of detail previously unachievable. SBF-SEM confirmed a disparity between cytoplasmic and nuclear P10 formation that remained throughout infection. Cytoplasmic P10 structures displayed a shift from thin angular structures that condensed to form thicker fibrous structures, which surrounded the nucleus. Due to the timing of this event it is suggested that this 'cage' structure could play an important role in nuclear lysis. SBF-SEM has also allowed 3D modelling of the EDS formation, showing the extent of the association with polyhedra, often resulting in the complete encasement of the polyhedra during the later phases of infection and possibly

acting as a precursor to the complete formation of the polyhedral envelope. This technique offers not only greater insight into the structure of P10 but also the functional role it plays during virus infection.

Contributed paper. Monday, 14:45, **19**

The Autographa californica multiple nucleopolyhedrovirus ac54 gene is crucial for the localization of the major capsid protein VP39 at the site of nucleocapsid assembly

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Baculovirus DNAs are synthesized and inserted into preformed capsids to form nucleocapsids at a site in the infected cell nucleus termed the virogenic stroma. Nucleocapsid assembly of Autographa californica multiple nucleopolyhedrovirus (AcMNPV) requires the major capsid protein VP39 and 9 minor capsid proteins, including VP1054. However, how VP1054 participates in nucleocapsid assembly remains elusive. In this study, the VP1054-encoding gene (*ac54*) was deleted to generate the *ac54* -knockout AcMNPV (vAc54KO). In vAc54KO-transfected cells, nucleocapsid assembly was disrupted, leading to the formation of abnormal elongated capsid structures. Interestingly, unlike cells transfected with AcMNPV mutants lacking other minor capsid proteins, in which capsid structures were distributed within the virogenic stroma, *ac54* ablation resulted in a distinctive location of capsid structures and VP39 at the periphery of the nucleus. The altered distribution pattern of capsid structures was also observed in cells transfected with AcMNPV absent of BV/ODV-C42 or cytochalasin D-treated AcMNPV-infected cells. BV/ODV-C42, along with PP78/83, has been shown to promote nuclear F-actin formation, which is another requisite for nucleocapsid assembly. Immunofluorescence using phalloidin indicated that the formation and distribution of nuclear F-actin was not affected by *ac54* deletion. However, immunoelectron microscopy revealed that BV/ODV-C42, PP78/83, and 38K failed to integrate into capsid structures in the absence of VP1054. Our findings suggest that VP1054 plays an important role in the transport of capsid proteins to the nucleocapsid assembly site prior to the process of nucleocapsid assembly.

Contributed paper. Monday, 15:00, **20**

Structural and functional analyses of the sulfhydryl oxidase P33 of Autographa californica multiple nucleopolyhedrovirus

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The Autographa californica multiple nucleopolyhedrovirus (AcMNPV) *p33* encodes a flavin adenine dinucleotide (FAD)-linked sulfhydryl oxidase, which is necessary for budded virus (BV) production and occlusion body (OB) formation. Here, we studied the structural and functional relatedness of P33 by making a serial of P33 point-mutants and studying their crystal structures and biological functions. P33 mutants of three conserved regions, including the FAD binding region, dimmer interface and R127-E183 salt bridge, were constructed. *In vitro* sulfhydryl oxidase activity tests of the purified proteins showed that the mutants of the FAD binding region and dimmer interface severely impaired the sulfhydryl oxidase activity of P33. The mutants of R127-E183 salt bridge, however, did not affect the sulfhydryl oxidase activity. The structures of wild-type AcP33 and a salt bridge mutant (R127A-E183A) present a preferable flexibility at the active site than the mutants of the FAD binding region and dimmer interface. Recombinant bacmids were constructed and infective viruses of all the mutants could be obtained except the mutants of the enzymatic activity sites. During the infection of the recombinant viruses, no obvious impacts on BV production and OB morphogenesis were observed. Currently we are working on bioassay to further investigate the effects of the mutants on larva infection.

Contributed paper. Monday, 15:15, **21**

Functional analysis of the conserved cysteines of AcMNPV GP41

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GP41 is a tegument protein of Autographa californica multiple nucleopolyhedrovirus (AcMNPV) and a thermosensitive gp41 mutant resulted in single-cell infections. To better analyze the function of GP41, a gp41 knock-out AcBacmid was constructed. Transfection-infection assay showed that GP41 was essential for infectious budded virus (BV) production. Electron microscopy showed that deletion of gp41 didn't have an obvious impact on nucleocapsids assembly, however, the egress of nucleocapsids from nucleus and the morphogenesis of occlusion-derived virus (ODV) were substantially blocked. The high-molecular weight oligomers of AcMNPV GP41 could be detected by Western blot analysis under non-reducing condition, suggesting that disulfide bonds may be important for GP41 function. Sequence alignment reveals that 5 cysteines (C91, C104, C125, C222 and C360) are conserved in GP41 of Group I aplhabaculoviruses. To investigate whether the disulfide bonds contribute to the structure and function of GP41, five cysteine single-site mutants were constructed. Among these mutants, C104G, C222G and C360G didn't impair BV production and GP41 oligomerization but ODVs are immature. C91G mutant produced lower amounts of infectious BVs at the early stage of infection, but the production of BV caught up with the level of wild type (WT) control virus at the late phase of infection. The oligomerization and ODV of C91G were found to be similar to those of the WT control virus. The C125G mutant was lethal for infectious BV production, and the morphogenesis of ODV was blocked to a premature stage. These results indicate C125 is a key cysteine for both BV and ODV formation of AcMNPV GP41.

Autographa californica multiple nucleopolyhedrovirus (AcMNPV) PIF protein AC83 is required for nucleocapsid assembly for both ODV and BV as well as recruitment of the PIF complex to the ODV envelopes

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The occlusion derived virions (ODVs) of baculoviruses initiate infection of lepidopteran larval hosts by binding to the midgut epithelia. ODV binding is mediated by the *per os* infectivity factors (PIFs). The AcMNPV PIF complex includes PIF1, PIF2, PIF3, PIF4, AC83 (P95) and the associated proteins PIF0 (P74), PIF5 (ODV-E56) and PIF6. Based on mass spectrometry data the PIF complex may also include AC108 and AC5. Stable PIF complex formation appears to require the interaction of PIF1-4, which forms the core upon which the other factors associate. Deletion of any of the PIF genes results in ODVs that are unable enter midgut cells. The exact function of each of the PIF proteins still remains to be determined and this study further analyzed the role of AC83. AC83 contains, like all PIF proteins, a transmembrane domain, as well as a zinc-finger domain, a predicted chitin binding domain, and a proline rich domain. The OpMNPV homologue was shown to be associated with both ODV nucleocapsids and membranes, but more recent studies reported that AC83 is only found in the ODV membranes. However, deletion of *ac83* eliminates nucleocapsid formation. Due to these discrepancies, we hypothesized that AC83 is required for nucleocapsid assembly for both ODV and BV, and that it may play a role in the PIF complex assembly. Detailed fractionation of ODV showed that AC83 is associated with both nucleocapsid and ODV envelopes. In addition, through the use of bacmids and extensive deletion analysis we provide additional definition of the AC83 domain structure and of a nucleocapsid assembly domain. We also demonstrate that AC83 is required for the recruitment of the PIF complex to ODV envelopes and thus enabling infection of insect midguts.

The host specificities of baculovirus *per os* infectivity factors

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Baculoviruses generally have narrow host ranges. The successful primary infection of baculovirus is initiated by the proper interaction of at least 7 conserved *per os* infectivity factors (PIFs) with the host's midgut cells, a process that remains largely a mystery. In this study, we investigated the host specificities of four core components (P74, PIF1, PIF2 and PIF3) of the PIF complex, using Helicoverpa armigera nucleopolyhedrovirus (HearNPV) backbone. The four PIFs of HearNPV were replaced by their counterparts from a Group I Autographa californica multiple nucleopolyhedrovirus (AcMNPV) or a Group II Spodoptera litura multiple nucleopolyhedrovirus (SpltNPV). Transfection-infection assays showed that all the pseudotyped viruses were able to produce infectious BVs. The expression and subcellular localization of the heterologous PIFs in HzAM1 cells were detected to be correct. Electron microscopy revealed that the formation and occlusion of ODVs of these pseudotyped viruses looked similar to the control viruses. Preliminary larval bioassay demonstrated that although all the pseudotyped BVs were lethal to *H. armigera* larvae via intrahaemocoelic injection, feeding larvae with very high concentration of occlusion bodies failed to kill the larvae except SpltNPV pif3 pseudotyped pif 3-null HearNPV (vHaBacΔpif 3-Sppif 3-ph). Further bioassay experiments showed that the LC50 value of vHaBacpif 3-Sppif 3-ph was 23-fold higher than that of the control virus vHaBacpif 3-Hapif 3ph, indicating that SpltNPV pif 3 can only partially substitute for the function of HearNPV pif 3. These results suggested that most of PIFs have strict host specificities, which may be responsible, at least in part for the limited host ranges of baculoviruses.

CONTRIBUTED PAPERS — Monday, 14:00-16:00 - *Chinon*

Bacteria 1 - *Ken Narva & Peter Kupferschmied*

Immunomodulatory activity of *Brevibacillus laterosporus* on the house fly

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Brevibacillus laterosporus is a bacterium morphologically featured by a typical canoe-shaped parasporal body and biologically characterized by broad-spectrum insecticidal and antimicrobial properties. There are numerous studies reporting the implication of different strains and of the toxins they produce in a post-ingestion insecticidal action involving disruptive effects on the midgut, as commonly observed for other bacterial entomopathogens. In the case of the house fly, some *B. laterosporus* strains affect also the insect physiology and development when administered at sub-lethal concentrations. To study the physiological implications deriving from the interactions between the house fly and *B. laterosporus*, we determined the main variations in the expression level of different immune-related genes by RT-qPCR analyses on the gut, fat body and haemolymph of house fly adults and larvae at different time intervals after *B. laterosporus* spore ingestion. Significant immune-modulatory effects of treatments were observed for diverse antimicrobial peptide (AMP) genes including *attacin*, *cecropin*, *defensin*, *domesticin* and *muscin*, and for *MdHSP-70*, *prophenoloxydase*, and *lysozyme*. An additional increase in *B. laterosporus* entomopathogenic action was observed when flies were previously immune-impaired through treatment with the plant-derived terpenoid compound azadirachtin. In summary, our study highlights the immunodeficiency potential of *B. laterosporus* when administered at appropriate dosage, thus providing new insights in understanding the physiological response of insects exposed to this bacterium.

Immune and detoxification systems of Colorado potato beetle infected with bacteria *Bacillus thuringiensis*

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Bacteria *Bacillus thuringiensis* var. *tenebrionis (Bt) are* naturally occurring bacterial disease and effective agent for biological control of important pest Colorado potato beetle (*Leptinotarsa decemlineata*). In this study we used Siberian population of Colorado potato beetle collected from the field that had never been sprayed with *Bt* -based biopesticides. Insects were infected by crystal toxins and spores of *Bt* in mix and separately. To highlight the question about insect defence reactions to *Bt*, immune and detoxification system as well as for intestinal microbiota composition have been tested in infected insects. To identify the bacterial communities of the intestinal microbiota of beetle larvae we used both method of bacteria cultivation on artificial media and the 16S sequencing. We found that bacteriosis led to dramatic change in midgut microbiota community, suppressed cellular immunity and induced detoxification processes in different tissues of insects. These findings will be discussed for understanding of the role of *Bt* vegetative cells and Cry toxins in infection process of Colorado potato beetle.

Immune priming might have evolved from infection by Gram+ bacterial pathogens in the mealworm beetle, *Tenebrio molitor*.

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The immunological experience of insects can improve their immune response or that of their offspring to subsequent infection. Such a phenomenon of immune priming has likely evolved from repetitive challenges by local microbial pathogens in the environment. Such a scenario might have favoured the evolution of certain level of specificity to microbial pathogens that represent the most important pathogenic threat in the local environment. In particular, the priming response to these microbes should be more efficient and less costly than the one produced against unfamiliar microbes. To test this hypothesis, the specificity of immune priming within and across generation was examined in the mealworm beetle, *Tenebrio molitor*, by comparing survival of individuals to infection with bacteria according to their own immunological experience or that of their mother with these bacteria. We found that primed individuals with Gram-positive bacteria became highly protected to both Gram-positive and Gram-negative bacteria compare to primed individual with Gram-negative bacteria. The offspring of primed females either with Gram-positive or Gram-negative bacteria exhibited similar levels of immune protection to all kind of bacterial infection. However, offspring from primed mothers with Gram-negative bacteria showed prolonged larval development than those of primed mothers with Gram-positive bacteria. These results suggest that whereas *T.molitor* is able to develop some levels of primed response to Gram-negative bacteria, immune priming might have particularly evolved from repetitive infections by Gram-positive pathogens, confirming that the latter bacteria might represent the most important pathogenic threat in this insect species.

Identification of synergist interactions between midgut bacteria of *Lymantria dispar* larvae and *Bacillus thuringiensis* HD-1

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Gypsy moth (*Lymantria dispar*) outbreaks can cause severe damage to forestry across Europe. In order to control this pest, there is need for new insecticidal bacterial strains for the development of more effective biopesticides. Mixed infections of insect larvae by pathogenic and non-pathogenic strains have been suggested to have an important role in maintaining toxin-based virulence and can even increase virulence (Raymond et al. 2007). The aim of this study was to characterize the bacterial midgut community of *L. dispar and* to screen for new biocontrol agents with synergistic activity against insect pests. For bacterial community analysis a cultivation dependent and a cultivation independent approach (16S rRNA gene sequencing using Single Molecule, Real-Time (SMRT) DNA Sequencing) was used. We further tested isolates from the *L. dispar* midgut community in combination with *Bacillus thuringiensis* strain HD1 in order to see if treatments with a mix consisting of a known biocontrol agent and midgut isolates can lead to increased larval mortality. We found that the tested midgut bacteria of *L. dispar* vary in their interaction with the pathogen. There are indications that some combinations can indeed increase the effectiveness of the applied biocontrol agent against *L. dispar*. This project was conducted within the frame of the SCIEX program with ETH Zurich as partner.

Plasmid-borne rap-phr systems control sporulation of *Bacillus thuringiensis* in insect larvae

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Bacillus thuringiensis (*Bt*) is an entomopathogen bacterium capable to colonize insect larvae, which are a privileged ecological niche. *Bt* harbors several plasmids which may be considered as adaptive factors for bacteria in their hosts. The lifecycle of *Bt* in the insect host is tightly controlled by quorum-sensing systems (PlcR-PapR, NprR-NprX and Rap-Phr) belonging to the RNPP family. The sporulation process, which is controlled by the master regulator Spo0A, ensures the survival and the dissemination of the bacteria in the environment at the end of the infectious cycle. The Rap proteins negatively affect the phosphorylation of Spo0A, thus preventing sporulation. These proteins are regulated by the Phr signaling peptides that directly inhibit their activity. Here, we investigated the role of plasmid-borne *rap-phr* genes on sporulation of the *Bt* HD73 strain in insect. Three *rap-phr* genes were found in pHT77, pAW63 and pHT8 1 plasmids. We showed that only the Rap from the pAW63 and pHT8 1 negatively affect the sporulation *in vitro*. We also characterized the Phr active forms needed to inhibit Rap activity. We demonstrated that the peptides AHGETI and AHGKDI, corresponding to the six N-terminal amino acids, were the minimal active forms of the Phr from pAW63 and pHT8 1, respectively. Finally, we showed that the *phr* mutants of pAW63 and pHT8 1 present a sporulation defective phenotype in insect larvae compared to the *rap -phr* mutant and to the wild-type strains. Our results demonstrate, for the first time, the involvement of plasmid Rap-Phr systems on the regulation of the sporulation process *in vivo*. Altogether our results show that insect larvae are an interesting and complex environment to study the role of plasmids in adaptive properties.

Contributed paper. Monday, 15:15, **29-STU**

A bioassay method to determine the insecticidal activity of *Bacillus thuringiensis* against *Ceratitis capitata* (Diptera: Tephritidae) and *Drosophila suzukii* (Diptera: Drosophilidae)

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Fruit flies are considered to be highly invasive polyphagous pests that cause serious damage to many crops worldwide. In the Mediterranean region, *C. capitata* (Wiedemann) and *D. suzukii* (Matsumura) are of major economic importance. *Bacillus thuringiensis* (Berliner) (Bt) has been considered as a potential source of insecticidal proteins for the control of *C. capitata*. However, bioassay methods used in previous studies have been hampered due to difficulties of knowing the number of individuals that consumed experimental inocula and issues related to quantifying the dose ingested by adult flies. We aimed to develop a novel, reproducible bioassay method for *C. capitata* and *D. suzukii*. This method uses a liquid colorant added to a food substrate that allows the identification of inoculated adults. It was also possible to determine the volume ingested in order to calculate lethal dose relationships. We demonstrate the value of this bioassay method using *Bt* strains, and a specific *Bt* protein (Cry2Ab25), which showed activity against *Ragolethis cerasi* (Tephritidae).

Contributed paper. Monday, 15:30, **30-STU**

Susceptibility of *Grapholita molesta* (Busck, 1916) to *Bacillus thuringiensis*, individual toxins and their mixtures

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The Oriental fruit moth, *Grapholita molesta* (Lepidoptera: Tortricidae), is a major pest of tree fruits worldwide, such as peach and apple. *Bacillus thuringiensis* has been shown to be an efficient alternative to synthetic insecticides in the control of insect pests. One objective of this study was to evaluate, for the first time, the effectiveness of *B. thuringiensis* in the control of *G. molesta*. Cry1Aa, Cry1Ab, Cry1Ac, Cry1C, Vip3Aa, Vip3Af, Vip3Ca, and the commercial products Dipel and Xentari were tested against *G. molesta*. Except for Cry1Ab and Vip3Ca, the rest of proteins/products tested were highly toxic to this insect species, with Dipel, Cry1Aa and Vip3Aa being the most active ones. To prevent or delay the development of resistance to Bt-crops the use of pyramided plants expressing multiple insecticidal proteins is highly recommended. Since Cry and Vip3 proteins have different midgut targets and different mechanisms of toxicity, Bt-crops combining these two types of toxins have already been marketed. However, few studies have focused on potential synergistic or antagonistic interactions between these types of toxins. As a second objective of this work we have evaluated the interaction between Vip3Aa-Cry1Aa, Vip3Aa-Cry1Ac and Vip3Aa-Cry1C and, in the three cases, a moderate antagonism has been observed.

Contributed paper. Monday, 15:45, **31-STU**

Interactions between HepG2 and Parasporin-3

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The Bacillus thuringiensis human cancer cell-active toxin Cry41Aa, also known as parasporin3, has only been shown to exhibit cytocidal activity towards two cell lines, and is only active after being proteolytically cleaved. In order to understand this activation mechanism various mutations were made at the N-terminal or C-terminal region of the protein and the toxicity against both HepG2 and HL60 cell lines was evaluated. Our results indicate that only N-terminal cleavage is required for activation and that N-terminally deleted mutants show some toxicity without the need for proteolytic activation. Furthermore we have shown that the relative toxicity towards the two cell lines depends on the protease used to activate the toxin. ProteinaseK-activated toxin was significantly more toxic towards HL60 than trypsin-activated toxin whereas there was little difference between the two against HepG2. In an attempt to better understand the mechanism of action of Cry41Aa against these cells we have evolved resistance in HepG2 through repeated exposure to increasing doses of the toxin. We will present results in which morphological, physiological and genetic characteristics of the resistant cell line are compared with susceptible cells.

SYMPOSIUM OF THE FUNGI DIVISION — Monday, 16:30-18:30 - *Descartes*

How Fungi mediate protection against herbivores and plant pathogens

Nicolai Vitt Meyling & Maya Raad

Contributed paper. Monday, 16:30, **32**

Plant protection potential of entomopathogenic fungi as endophytes: What is the evidence and what is the mechanism?

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Since 2000, there has been increased focus on the potential of using endophytic entomopathogenic fungi, particularly *Beauveria bassiana,* for biological control against insect herbivores and plant pathogens. We reviewed the published literature for evidence of the biological control effects reported when various crops were inoculated with *B. bassiana*. Overall, *B. bassiana* as an endophyte appears promising for biological control having both negative and neutral effects on insect herbivores, however, there remains ambiguity with respect to the location and mode of action of the fungus. Detection of endophytes in plant tissues is problematic as it is method-dependent. There is a need for stringent protocols for surface sterilisation including thorough experimental controls, preferably combined with molecular detection techniques by PCR. Though having negative

effects against insects, mycosis is rarely reported and in those cases location of the infective units remains elusive. Overall, there is very little known about the mechanisms causing the documented non-pathogenic negative effects on herbivores. A future focus on the mode of action when entomopathogenic fungi associate with plants will greatly increase our understanding of the mechanisms behind reported negative effects against pests.

Contributed paper. Monday, 16:45, **33**

Microbial-induced resistance against herbivores: mechanisms and ecological consequences

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Beneficial microbes are present in all ecosystems and in all organisms. Plant-insect interactions as we have understood them, are actually shaped by microbes that interact with the plants, with the herbivores and with the carnivores, both below- and aboveground. In recent years scientists have discovered that the mechanisms that underlie these interactions are common for different microbes. One of those fascinating mechanisms is the microbial-induced systemic resistance (ISR) that changes plant physiology, affecting pathogens and herbivores. Even more fascinating is the fact that such physiological mechanism in the plant has important ecological consequences for different trophic levels. I will present how beneficial soil microbes - with a special focus on rhizobacteria - affect herbivores with different feeding behaviour (i.e. aphids and caterpillars) and degree of specialization (i.e. specialists and generalists). But also how herbivore parasitoids respond to the presence of beneficial microbes, and whether this is mediated by changes in the emission of herbivore induced plant volatiles. We will discuss ideas on how we can use both mechanistic and ecological knowledge of microbe-plant-insect interactions to develop new strategies to control herbivore pests.

Contributed paper. Monday, 17:10, **34**

Priming of plant defenses against herbivores by arbuscular mycorrhizal fungi

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The effect of microbial communities on plant performance has been described extensively, and the stimulation of plant immunity by beneficial microbes is under extensive scrutiny. Arbuscular mycorrhizal (AM) fungi are obligate biotrophs widespread in natural and agro-ecosystems. They colonize the roots of most land plants to establish mutualistic associations with multiple benefits for the host: increased plant nutrition, enhanced tolerance to abiotic stresses and induced systemic resistance to biotic stresses both below and aboveground. During root colonization, the AM fungi have to deal with the plant's immune system, and as a consequence, plant defenses are primed. We show that the colonization implies a transcriptional and metabolic reprogramming of the roots that impacts hormone homeostasis, likely underlying the enhanced ability to cope with multiple stresses. Mycorrhizal plants, however, do not show major alterations of basal levels of defense related phytohormones in shoots, but their accumulation is boosted upon attack. Alterations in the volatile profile upon herbivory and enhanced attraction of natural enemies has also been shown. Using tomato as a model, we analyzed the impact of the mycorrhizal symbiosis on the generalist *Spodoptera exigua* and the specialist *Manduca sexta*. Survival of the herbivores was significantly lower when feeding on mycorrhizal plants, and this reduced performance correlated with a primed accumulation of jasmonic acid (JA) and absicic acid (ABA) and related defense genes. This mycorrhiza induced resistance was abolish in tomato mutants impaired on JA and ABA signaling pathways, confirming the relevance of those pathways in the protection observed.

Contributed paper. Monday, 17:35, **35**

Induced systemic resistance by *Trichoderma spp*

Christine Vos[1,2,3], Katrijn Raymaekers[1,2], Yuxia Yang[1], Kaat De Cremer[1,2], Barbara De Coninck[1,2], Kemal Kazan[3], Bruno Cammue[1,2]
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The genus *Trichoderma* constitutes a promising collection of potential biocontrol organisms, reducing plant disease either via direct interaction with plant pathogens and/or indirectly through induced systemic resistance (ISR). This cost-effective mechanism allows the plant to react faster and stronger to a subsequent pathogen attack. In our group research has focused for some years on the induced systemic resistance response triggered in plants by colonization with *Trichoderma* spp. We have investigated the transcriptome response of *Arabidopsis thaliana* and tomato plants to *Trichoderma harzianum* T382 in interaction with the grey mould pathogen *Botrytis cinerea* and the vascular wilt pathogen *Fusarium oxysporum* in order to unravel the underlying mechanisms of ISR. In addition, a screening platform has been set up based on marker genes to screen for ISR-inducing capacity in micro-organisms and compounds. Recent research also includes the ISR-inducing capacity of *Trichoderma* culture filtrates, and looks into the possibility of triggering ISR from the leaves instead of the roots. Our recent developments will be discussed in the presentation.

Contributed paper. Monday, 18:00, **36**

Elucidating the mechanisms of *Beauveria bassiana* induced plant resistance

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Beauveria bassiana can adopt an endophytic lifestyle by colonising a wide array of plant species. Several studies have reported enhanced resistance against insects and plant pathogens from colonised plants. However, little is known about the molecular mechanisms that govern such interactions or explain any resistance effects. Elucidating the molecular responses of the plant is therefore needed and will help to better understand this little known aspect in the ecology of entomopathogens. We carried out a genome wide expression analyses of *Arabidopsis thaliana* plants colonised by the *B. bassiana* strains BG11 and FRh2 after root inoculation. The analyses suggest that both *B. bassiana* strains evoked a microbe-associated molecular pattern triggered immunity (MTI) in *Arabidopsis* leaves 15 days post infestation. This MTI resulted in the induction of jasmonic (JA), ethylene (ET) and salicylic acid (SA) signalling pathways genes in BG11 colonised *Arabidopsis*. However, this induction did not result in higher levels of JA and SA suggesting priming as a possible mechanism. Analyses also showed that *Arabidopsis* interaction with both strains resulted in the induction of the antimicrobial compound camalexin and multiple reactive oxygen species scavengers such as peroxidases and glutathione-S-

transferases. Based on this, we tested whether *B. bassiana* colonisation enhances plant resistance against the pathogen *Sclerotinia sclerotiorum*, which is known to be sensitive to JA/ET and SA induced defences and to camalexin. Treatment of *Arabidopsis* roots with both strains significantly decreased leaf lesion size caused by *S. sclerotiorum*. To our knowledge this is the first study to assess the transcriptome of a plant colonized by *B. bassiana.*

CONTRIBUTED PAPERS — Monday, 16:30-18:30 - ***Courteline***

Diseases of Beneficial Invertebrates 1 *- David Bass*

Contributed paper. Monday, 16:30, **37-STU**

Flat oyster follows the apoptosis pathway to defend against the protozoan parasite *Bonamia ostreae*

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The protozoan *Bonamia ostreae* is an interesting model to investigate the interactions between oysters and parasites at the cellular level. Indeed, this unicellular parasite infects the flat oyster *Ostrea edulis* and multiplies within hemocytes, the central effectors of oyster defenses. Apoptosis is a mechanism used by many organisms to eliminate infected cells. In order to study the potential involvement of this mechanism in the oyster response to *B. ostreae*, *in vitro* experiments were carried out by exposing hemocytes from the naturally susceptible oyster *O. edulis* and a resistant oyster species *Crassostrea gigas* to live and heat-inactivated parasites. Hemocyte apoptotic response was measured using a combination of flow cytometry and microscopy analyses. Whatever the host species was, the parasite was engulfed in hemocytes and induced an increase of apoptotic parameters including intracytoplasmic calcium concentration, mitochondrial membrane potential or phosphatidyl-serine externalization as well as ultrastructural modifications. However, the parasite appears more able to infect flat oyster than cupped oyster hemocytes and the apoptotic response was more important against live than dead parasites in the natural host than in *C. gigas*. Our results suggest that *O. edulis* specifically responds to *B. ostreae* by inducing apoptosis of hemocytes.

Contributed paper. Monday, 16:45, **38**

Influence of temperature on the haplosporidian parasite *Bonamia ostreae* exposed to *Crassostrea gigas* and *Ostrea edulis* oyster mucus

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The increase of temperature in seawater due to climate change could produce an adverse impact in fisheries worldwide. The adaptation of the animals to the new habitat can increase the incidence of disease, parasites and competitors. The body surface of mollusks is covered by a layer of mucus which is involved in several functions included defense against infectious agents. Mucus is composed by mucins and a wide range of bioactive molecules that constitute the first barrier against pathogens organisms. In this context, the effect of mucus from the susceptible oyster *O. edulis* and the resistant oyster *C. gigas* to the haplosporidian parasite *B. ostreae* was evaluated. *B. ostreae* was exposed to 1 mg/mL protein mucus of each species at 10, 15, 20 and 25°C for 3 hours and parasite mortality was measured by flow cytometry. In order to investigate if mucus modifies subsequent interactions between parasites and oyster defense mechanisms, mucus-exposed *B. ostreae* was also confronted with hemocytes. Percentage of phagocyted parasites was evaluated microscopically and the expression of parasite genes HSP90, Actin-1 and 2, GAPDH and 18S was measured by qPCR. Significant differences in mortality were observed after exposing *B. ostreae* against mucus of the two oyster species. Interestingly, percentage of mortality was higher in *B. ostreae* exposed to *O. edulis* mucus than *C. gigas* mucus suggesting an adaptive response to parasite infection. No significant differences in mortality rate among the four temperatures tested were observed. A lower percentage of phagocytosis was observed in parasites challenged than control ones suggesting that mucus can interfere with parasite infection mechanisms. Gene expression of parasite will be discussed afterwards.

Contributed paper. Monday, 17:00 **39-STU**

Monitoring of the autophagy pathway in *C. gigas* during an experimental OsHV-1 infection at cellular molecular and proteomic levels

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Recent mass mortality outbreaks around the world in Pacific oysters, *Crassostrea gigas*, have seriously affected the aquaculture economy. Although the causes for these mortality outbreaks appear complex, a viral agent has been identified as a main factor: the virus ostreid herpesvirus1 (OsHV-1). A comparison between human and oyster genomes indicate that proteins are conserved between the two species, especially proteins implicated in autophagy. Autophagy is an important degradation pathway involved in several pathologies including infectious diseases. Numerous lines of evidence suggest that autophagy plays a key role in the clearance of many viruses. Recent results indicate that an autophagy pathway is functional in the Pacific oyster and autophagy is involved during OsHV-1 infection. According to recent work, the aim of the present study was to investigate the autophagy pathway in *C. gigas* in presence of OsHV-1 to better understand the process of autophagy related to immune response of the Pacific oysters during the viral infection. For this purpose, experimental infections by OsHV-1 injection were carried out on families of Pacific oysters. The autophagy pathway response was studied using different approaches. At cellular level, flow cytometry was used to monitor autophagosomes formation in haemocytes during the time of the experiment. At molecular level, the expression of viral genes and autophagy genes was followed using RT PCR. The proteomic

level was finally explored by assessing the expression of the key protein marker of the autophagy pathway, LC3. The first results showed an activation of the autophagy pathway in the heamocytes 14 hours after virus injection in oysters.

Contributed paper. Monday, 17:15, **40**

Genome sequencing of the Ostreid herpesvirus 1 infecting oysters in Tomales Bay, California

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An emerging infectious disease of oysters is the Ostreid herpesvirus 1 (OsHV-1) which is spreading globally. The OsHV-1 and its variants, are virulent and problematic viruses of larval, seed, and adult oysters. OsHV-1 was fully sequenced and characterized as a member of the Family *Malacoherpesviridae*. Multiple strains of OsHV-1 exist and may vary in virulence, i.e. OsHV-1 μvar. Understanding genome variability may be key in developing control mechanisms and improved diagnostic tools for limiting spread of global OsHV-1 strains. For most global variants of OsHV-1, gene or genome sequence data is limited to PCR-based sequencing. In the US, OsHV-1 has been observed only in 2 California bays, Tomales Bay and Drakes Estero, since the early 1990s where it causes severe (50-60%) losses of oyster seed most summers. In order to better understand both strain variation of OsHV-1 infecting oysters in Tomales Bay, we generated meta-genomic data (Illumina MiSeq) from individual infected oysters (n=4 per year) collected in 2003, 2007, and 2014. Nearly complete California OsHV-1 genome sequences and low overall microbial diversity were achieved from highly infected oysters. Increased microbial diversity was detected in three of four samples sequenced from 2003, where genome copy numbers were lower. Comparisons of the California strain of OsHV-1 to available full genome indicate the genome is similar but not identical to one sequenced strain of OsHV-1. Data from our metagenomic sequencing support previously collected transcriptomic data for the California OsHV-1. Taken together, our results indicate that meta-genomic or transcriptomic sequencing may be a powerful tool in understanding strain variation of non-cultivated pathogens.

Contributed paper. Monday, 17:30, **41-STU**

Reducing the impact of pathogens and disease in the Irish Pacific oyster *Crassostrea gigas* by understanding Environment: Host/Pathogen interaction

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Pacific oysters *Crassostrea gigas* have been farmed in Ireland since the 1970's. However, since 2008 oyster mortality events have been associated with herpesvirus infection, in particular the variant OsHV-1 μVar, and pathogenic strains of the bacterium *Vibrio aesturianus.* This study is looking at practical control measures to reduce the impact of oyster pathogens and to better understand how these pathogens might sustain themselves outside the host in the marine environment. Environmental factors have a significant impact on pathogen and disease development. In this study the effect of salinity on pathogen development in *C. gigas* at high and low/variable salinity was assessed in relation to the oyster's ability to grow while resisting infection. The field trial was conducted in 2015 in Carlingford Lough, Immune function of oysters was better when oysters were held at lower salinity regimes. Digestive gland condition indicated poor feeding circumstances for both salinity sites. Sexes were expected to be undetermined (triploid), but a high percentage of males were found in all samples. Growth rates did not differ for the same oyster age groups between low and high salinity site. The overall prevalence of herpesvirus was low (< 5%) in oysters at both salinities for both age groups and mortalities were minimal. These results were attributed to the lower than average seawater temperatures (< 16°C) experienced, which when elevated act as a trigger/stressor for disease outbreak. Results from the field trials would indicate that active husbandry and movement of oysters between areas of different salinity during the culture cycle can maximize growth and immune capability and minimize pathogen and disease impact.

Contributed paper. Monday, 17:45, **42**

Apicomplexans infecting marine molluscs

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Numerous apicomplexans infecting marine molluscs have been described and there are an increasing number of molecular sequences available in the databases. However, molecular phylogenetic analyses are not consistent in the placement of these sequences and their higher-level taxonomic position is not resolved. In addition, there remains some ambiguity as to which life stages should be present in the life cycles, and whether more than one host is utilized, such as the differences between the genera *Pseudoklossia* (heteroxenous) and *Margolisiella* (monoxenous). Histological and molecular studies have now demonstrated that two mollusc hosts, a gastropod and a bivalve, are infected by a single species of apicomplexan, and that they are highly likely to represent a obligate life cycle. Molecular phylogenetic analyses reveal that the majority of apicomplexans infecting marine invertebrates form a well-supported clade and probably represent a discrete new sub-order that includes the order Agamococcidiorida and family Aggregatidae. These findings suggest that we still have a relatively poor understanding of all the life stages present in some apicomplexans parasites and which we might expect to find in what host or hosts.

Contributed paper. Monday, 18:00, **43**

Is an apicomplexan responsible for the collapse in the Iceland scallop stock in Iceland?

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Since 2003, a commercial fishing ban has been in force for Iceland scallop, *Chlamys islandica*, in Icelandic waters due to a total and unexpected collapse on the stock. Following the initial identification of apicomplexan infections in the scallops, a long-term surveillance program was established to evaluate the effect of the parasite on the population. Infections were highly prevalent throughout the study but only affected mature scallops where they caused severe macroscopic changes. A significant relationship was observed between infection

intensity and the condition of both gonads and adductor muscles. The first four years of the study, were characterized by high infection intensity and very poor condition of the adductor muscle and gonads, followed by a gradual decrease in infections and improvement of the condition of the scallops. Histopathological examination showed that infections were widely distributed and caused varying degrees of pathology in muscular and connective tissues of most organs. The progression of the infections was in good synchrony with the mortality rates and the decline observed in the stock and recruitment indices. Our findings strongly suggest that this apicomplexan parasite played a major role in the collapse of the scallop stock around Iceland. Furthermore, the infections had significant impact on gonad development, which contributed further to the collapse of the stock in the form of lower larval recruitment. Compelling evidence exists that this apicomplexan pathogen is also causing disease outbreaks in other scallop populations as similar macroscopic changes, and the parasite itself, have been observed in association with mass mortality events in several different scallop species.

CONTRIBUTED PAPERS — Monday, 16:30-18:30 - *Vouvray*

Virus 2 - *Robert Harrison & Bergmann Ribeiro*

Contributed paper. Monday, 16:30, **44-STU**

Genome stability of AgseNPV-B after serial *in vitro* passages

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Natural isolates of baculoviruses represent polymorphic populations as they consist of genetically diverse individuals. Albeit these individuals may interact in infections, they also undergo natural selection through hosts and tissues infected in the same time. One scenario are serial passages of baculoviruses in cell lines, as a specific selection pressure different from *in vivo* infections is put on the genome. Lately, infections of an Agrotis segetum nucleopolyhedrovirus B isolate (AgseNPV-B) in larvae of *Agrotis segetum* were described in dose-response bioassays and with molecular biological methods. Further, budded virus suspensions of AgseNPV-B produce polyhedral inclusion bodies in the permissive cell line AiE1611T. Therefore AgseNPV-B is a promising candidate for the *in vitro* production of a biological control agent against cutworms. In order to study the stability and to gain insights into the correlation of virulence and genetic variation, a plaque purified clone of AgseNPV-B was serially passaged ten times through AiE1611T cells. Selected passages were compared in dose-response bioassays with L2/L3 larvae of *A. segetum* and genomic DNAs of these virus passages were deep-sequenced resulting in average 2,500-fold genome coverage. Variations were revealed with mutations in several baculovirus core genes and homologous repeat regions. The *in vivo* infectivity was reduced following 10 serial *in vitro* passages. The hotspots of genetic variability and the associated virulence are crucial findings for any *in vitro* production of AgseNPV-B since it demands continuous quality control of *in vivo* virulence of baculovirus biocontrol agents produced in cell cultures.

Contributed paper. Monday, 16:45, **45-STU**

Synthetic baculovirus genomes to extend host range

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We have previously reported the successful synthesis of the first baculovirus, AcMNPV-WIV- syn1.0 that retained the properties of wild-type AcMNPV. Here we explored the same technique to study factors related to the host range of baculoviruses. AcMNPV and BmNPV are closely related with about 95% genome identity. However, AcMNPV is unable to infect *Bombyx mori.* In order to achieve an AcMNPV which can infect *B. mori,* a series of synthetic AcMNPV genomes were constructed in which i) *helicase*; ii) *helicase* and *lef3*; iii) *pif* s *0-6*; iv)*helicase* and *pif* s *0-6*; v) *helicase*, *lef3* and *pif* s 0-6; vi)*helicase*, *pif* s *0-7*, *vp91* and *Ac5*, and vii) *helicase*, *lef3*, *pif* s 0-7, *vp91* and *Ac5* were replaced by their BmNPV homologs. The genomes were synthesized by three rounds of transformation-associated recombination in *Saccharomyces cerevisiae* as previously reported and used to transfect Sf9 cells to rescue progeny budded viruses (BVs). One step growth curve assays showed that all 7 synthesized viruses replicated productively in Sf9 cells, and 6 of which (except the virus with pifs 0-6 replaced by BmNPV pif genes) also grow well in BmN cells. BVs harvested from BmN cells also killed *B. mori* larvae by intrahaemocoelic injection. We are currently testing the oral infectivity of the synthetic viruses.

Contributed paper. Monday, 17:00, **46-STU**

Genotype Detection and Abundance within Baculoviruses using Next Generation Sequencing

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Next Generation Sequencing (NGS) generates short 'reads' of viral sequences between 75 and 500 base pairs (bp). We have developed a method that uses the Ion Torrent PGM to detect genotypes and relative abundance within a baculovirus isolate.
The software pipeline was developed to quantify baculovirus genotypes within Helicoverpa armigera SNPV isolate AC53. Genotypes and their relative abundance within isolates were determined from nucleotide based polymorphisms within NGS data of amplicons from BRO-A, HOAR and DNA polymerase open reading frames (ORFs). The method was subjected to a two-step validation using Sanger sequencing and analysis of relative abundance of strains derived from the parent strain by passage and plaque selection in tissue culture. The technique was then applied to determine changes in relative abundance of viral variants under selection *in vivo* and *in vitro*. Using the NGS data and an open-source software pipeline we can identify and determine genotype abundance within baculovirus populations with application to wider microbial metagenomic studies.

The complete genome sequence of Plodia interpunctella granulovirus: discovery of an unusual inhibitor of apoptosis (IAP) gene

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Plodia interpunctella granulovirus (PiGV) is a baculovirus that infects larvae of the Indianmeal moth, *Plodia interpunctella*. In addition to research on the use of PiGV to control *P. interpunctella* infestations of stored goods, the PiGV/ *P. interpunctella* virus/host pair has been developed into a system for investigating the ecology and evolution of infectious disease. To help inform research using PiGV, the complete genome was determined by next-generation sequencing of DNA isolated from occlusion bodies. The genome was found to be 112, 536 bp in length with a 44.2% G+C nucleotide distribution. A total of 123 open reading frames (ORFs) and seven homologous regions (hrs) were identified and annotated. Phylogenetic inference based on baculovirus core genes placed PiGV in the "b" clade of viruses from genus Betabaculovirus with a branch length suggesting that PiGV represents a previously unrecognized betabaculovirus species. A number of ORFs evidently acquired by horizontal gene transfer were documented in the PiGV genome. Among these genes, an ORF (ORF81) encoding an Inhibitor of Apoptosis Protein (IAP) homologue containing two Baculovirus Inhibitor of apoptosis Repeat (BIR) domains was identified which shared significant sequence similarity with insect cellular IAPs, but not with viral IAPs. The ORF81 product also resembled cellular IAPs in terms of its predicted size and the presence of a long N-terminal leader sequence. Phylogenetic inference with selected insect and baculovirus IAPs indicated that while some classes of baculovirus IAPs (IAP-1, IAP-5) are ancient lineages arising from a single gene acquisition event, PiGV ORF81 and member genes of the IAP-3 class are likely the result of multiple acquisitions which occurred more recently.

Study of the domestication of a viral genome in the parasitoid wasp *Venturia canescens*

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In the parasitoid wasp *Venturia canescens,* viral particles devoid of DNA are produced in the ovaries and secreted in the oviduct where they become attached to the eggs chorion. They are introduced with the eggs into the parasitized host during wasp oviposition. The particles called VLPs for Virus-Like-Particles protect the eggs from the encapsulation defense mechanism thus allowing the development of wasp larvae in parasitized caterpillars having an effective immune system. It has been recently shown that VLPs are produced by an endogenous nudivirus present in the genome of the wasp. VLPs are made of proteins of wasp origin wrapped into a viral enveloppe. A detailed bioinformatic analysis of the parasitoid genome reveals that among the essential genes of a nudivirus those that have been lost in *Venturia canescens* can however be detected as pseudogenes in the wasp genome. In particular we could identify remnants of genes coding for structural components of the nucleocapsid allowing DNA packaging, which would explain that VLPs do not contain DNA. Interestingly pseudogenization affects genes selectively; indeed some of the pseudogenes are located within clusters of nudivirus genes that are still active. Compared to bracoviruses also originating from a nudivirus endogenized in the wasp genome approx. 100 million years ago, *Venturia canescens* VLPs provide an insight on early events of virus domestication. Wasp genome analysis indicates that selection acting on individual genes rather than major rearrangements, such as a deletion of a large part of the virus genome, is the major driving force leading to loss of viral functions, such as DNA packaging.

Adaptation genomics in the bracovirus of *Cotesia sesamiae*

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The parasitoid wasps of the *Cotesia* genus are widely studied for their agronomical interest. The larvae of these wasps grow inside their host and have to overcome host defences. Among the wide array of strategies used by parasitoids, these wasps have developed one of the most original: the domestication of a virus, named bracovirus. This bracovirus has been stably integrated in the wasp genome and allows the wasps to produce particles containing virulence genes. These particles are introduced simultaneously with the wasp eggs in the host, induce immunosuppression and allow wasp larval development and parasitism success. *Cotesia sesamiae* is an African species and parasitize over twenty lepidopteran stem borer species. To investigate the role of the bracovirus in wasp local adaptation or specialization to their lepidopteran hosts, we focused on 25 samples representative of different African *C. sesamiae* populations and that constitute a continuum of speciation. We used custom-made targeted sequence capture to enrich our sequence library in bracovirus regions prior to Illumina resequencing. We used phylogenomic and population genetic tools to identify genes under divergent selection between populations. We have shown this region evolves very fast, with different genes under selection and structural genomic constraints. However, this study focused on the bracovirus does not give a global vision of the selection traces in the whole wasp genome. To answer that, we made a RAD-Sequencing approach to analyze the demographic history of these populations and to identify genomic regions that show a strong differentiation. These analyses increase our knowledge on the evolutionary processes that lead to speciation and how they shape the genomes.

Expansion of the family *Nimaviridae*

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White Spot Disease (WSD) is caused by the virus White Spot Syndrome Virus (WSSV 1) and is a notifiable disease to the World Organisation for Animal Health (OIE) and within Europe. All decapod crustaceans are listed as being susceptible to this disease and recent work has demonstrated that European decapods (e.g. crabs, lobsters, shrimp and crayfish) show widely differential susceptibility to WSSV 1. To date, WSSV 1 was the sole member of the genus *Whispovirus* within a very distinctive DNA virus family, the *Nimaviridae*. Since this is a relatively newly recognised viral family the International Committee on Taxonomy of Viruses (ICTV) acknowledge that this family is likely to expand as new viral taxa are discovered. Previous studies have identified viral infections in the shore crab (*Carcinus maenas*), in particular B virus and Rod shaped virus of Carcinus maenas (RVCM), and suggested that these may in fact be ancestral forms of WSSV 1. Vlak *et al*. (2005) tentatively listed these as putative members of the family *Nimaviridae*, however these listings were removed due to lack of evidence as it had not been possible to compare these viruses directly with WSSV 1 isolates from penaeid shrimp farming regions. We have recently re-isolated RVCM infecting shore crabs from Canadian waters. Histologically and ultrastructurally RVCM appears to be very similar to WSSV 1. However newly available genomic information for RVCM, derived from in-house MiSEQ analysis of infected tissues, has shown that RVCM appears to be the second member of the family *Nimaviridae*, with high similarity to WSSV 1.

Characterization and complete genome sequence of a new cypovirus isolated from *Thyrinteina arnobi* (Stoll, 1782) (Lepidoptera: *Geometridae*)

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Cypoviruses are non-enveloped dsRNA insect viruses from the family *Reoviridae* and have been found in insects from different orders including Lepidoptera, Diptera, Hymenoptera, and Coleoptera. A hallmark of cypoviruses is the formation of an occlusion body (OB) composed mainly of a protein called polyhedrin, which protects the icosahedral-shaped virions in the environment outside the insect host. In this work, a new virus found infecting larvae of *Thyrinteina arnobia* was characterized. This insect is the main lepidopteran defoliator pest of eucalyptus in Brazil. Larvae with symptoms of virus infection were collected from our laboratory colony and analyzed for the presence of virus particles. OBs were easily seen by light microscopy analysis of the insect extracts. These OBs were purified and further analyzed by Scanning and Transmission Electron Microscopy, confirming their viral nature. Nucleic acid extraction of purified OBs were analyzed by electrophoresis in agarose gels and showed the presence of 10 fragments of dsRNAs. These dsRNAs were then sequenced and the genome annotated. Phylogenetic analysis using the *polyhedrin* gene indicated that this virus clustered with the species *Cypovirus 14*. Moreover, the *polyhedrin* gene was then amplified by Polymerase Chain Reaction and cloned into a transfer vector that was then used for the construction of a recombinant baculovirus. The recombinant baculovirus was used to infect *Spodoptera frugiperda* cells (Sf9) and shown to produce OBs in the cell cytoplasm. Bioassays will be carried out in order to analyze the potential of this new virus as a biological control agent of this important insect pest.

CONTRIBUTED PAPERS — Monday, 16:30-18:30 - ***Chinon***

Microbial Control 1 - *Travis Glare*

Investigations on spore residues of the product XenTari® (*Bacillus thuringiensis* subsp. aizawai) and their persistence on sweet pepper and tomato

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The bacterium *Bacillus thuringiensis* (*Bt*) has been described by Ernst Berliner in 1915. It's an important part of biological pest control, because of its toxicity against the larvae of several insects. *Bt* -based insecticides have been used successfully over several decades. Because of one incidence of diarrhoea in 2012, where high concentrations of presumptive *Bacillus cereus* (including *Bt*) were found in lettuce samples in Germany a discussion about the risk of *Bt* residues started and is still ongoing.

For most bacteria threshold values for food are existing to ensure the consumers safety. This threshold value is enumerated as colony forming units (cfu) and is at 105 cfu/g food for presumptive *B. cereus*. Since it has been shown that *Bt* is capable of producing enterotoxins, the danger of goods treated with *Bt* -products is being analyzed. On this account we focused on spore residues on sweet peppers and tomato under professional glasshouse growing conditions. Within these experiments maximum application rates were used and therefore, these experiments represent a kind of "worst case" scenario. In most of the cases the threshold value of 105 cfu/g fresh weight was not exceeded. Similar results could be achieved during laboratory experiments with own applications. It was also shown that storage of samples at -20°C for at least three months was not influencing the cfus. A very plain experiment indicates the possibility to affect the exposition to residues on products.

Encapsulation and UV Photoprotection of a Vip3 toxin

Inigo Ruiz De Escudero[1,2], Leopoldo Palma[3], Francisco Mañeru[1], Primitivo Caballero[†1,2]

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Bacillus thuringiensis Vip proteins are secreted during the vegetative phase of bacteria growing. This characteristic with its rapid degradation upon exposure to solar ultraviolet radiation hinders its use as a biological insecticide. To avoid secretion, improve UV-protection and compare the influence of the protein source, the *vip3Ag4* gene was cloned into two heterologous bacteria. Vip3Ag4 was expressed and subsequently purified or encapsulated into a *P. fluorescens* strain and into a *Bacillus megaterium* strain. The toxicity of Vip3Ag4 protein from different sources was evaluated against *Spodoptera littoralis* larvae. Vip3Ag4 was produced in *P. fluorescens* and in *B. megaterium* and purified, after cell lysis, in an amylose affinity column using a maltose binding protein (*Pf* -MBP-Vip3Ag4) or in a Nickel affinity column using a Histidine Tag protein (*Bm* -HisTag- Vip3Ag4), respectively. Vip3Ag4 was produced in parallel and remained encapsulated after a Lugol's fixation in both cell types (*Pf* - Vip3Ag4 and *Bm* -Vip3Ag4). The protein was active at the same level against *S. littoralis* larvae, independently of the protein source. When purified Vip3Ag4 was exposed to UV radiation a 10-20-fold loss in activity was observed. However, when protein remained encapsulated the lack of activity was significantly lower, due to the photoprotection offered by *P. fluorescens* and *B. megaterium* encapsulation. Advantages of each expression system are discussed.

Genetic and biological characterization of *Bacillus thuringiensis* isolates showing insecticidal activity against *Leptinotarsa decemlineata* (Coleoptera: *Chrysomelidae*)

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The Colorado Potato Beetle (CPB) [*Leptinotarsa decemlineata* (Say) (Coleoptera: *Chrysomelidae*)] is the most important defoliator pest of potato crops worldwide. The development of resistance to virtually all groups of chemical insecticides, used to prevent or reduce damage from this pest, has increased the need for research into alternative methods that are effective and more sustainable. *Bacillus thuringiensis* (Bt) toxicity factors can be used for both the development of microbial insecticides and for the production of transgenic plants. Until date, there is only one commercial Bt product (serovar *morrisonis*) that produces Cry3 proteins to control beetle pests. This study aimed to identify *Bt* strains, toxic to species of the family Chrysomelidae (e.g. *L. decemlineata*), which have been selected for the presence of insecticidal genes against coleopteran species. The mortality caused by a single crystal protein concentration of the different Bt strains was determined in second instars of *L. decemlineata* in a preliminary leaf bioassay. The relative potency of Bt strains against *L. decemlineata* larvae neonate, was compared to that of the Bt strain used as the active ingredient in the commercial product Novodor®. We discuss the potential of each Bt strain as a control agent for beetle pests in view of the *cry* genes they carry.

Analysis of the occurrence of cry, vip3 and chitinases genes in *Bacillus thuringiensis* strains isolated from Algeria

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Bacillus thuringiensis is the most widely used bacterium in biocontrol as alternative for chemical insecticides. Its entomopathogenic features are the result of its capacity to produce a wide range of insecticidal proteins, both during its vegetative growth stage (Vip proteins) and during sporulation (Cry proteins). Based on the difference in targets of Cry and Vip proteins and the synergic action of chitinase enzymes, we set out to screen an Algerian collection of *B. thuringiensis* isolates for genes encoding entomopathogenic proteins with the aim to find isolates with high insecticidal activity and a broad range of action. A total of 75 *B. thuringiensis* strains were isolated from soil, grain storage and dead insects, based on the observation of parasporal crystals under light microscopy. The PCR screening of *cry1, cry2, cry9, vip3, exochitinase* and *endochitinase* genes rendered 38, 52, 42, 59, 40 and 51 strains, respectively, that gave amplicons with the expected size for their respective genes. Eight strains were positive for all six tested genes and were selected for subsequent bioassays with some lepidopteran pests. Depending on the expression of the carried genes, the selected strains may become excellent candidates for biocontrol and formulation of new biopesticides to control lepidopteran pests.

Increase toxicity from a modified Cry3Aa toxin against *Monochamus alternatus*

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Monochamus alternatus Hope is the main vector of pine wilt disease caused by pine wood nematode (*Bursaphelenchus xylophilus*) in China. Biological control is an effective method to manage pine wilt disease. *Bacillus thuringiensis* (Bt) toxins are widely used to control insect

pests. Although some Bt toxins, such as Cry3, Cry7 and Cry8, are highly toxic to coleoptera, no effective Bt toxins targeting *M. alternatus* have been identified. We previously tested a Bt toxin, Cry3Aa, which showed a low toxicity to *M. alternatus*. Preliminary data suggested that Cry3Aa cannot be effectively activated by the midgut proteases of *M. alternatus*, resulting in a low insecticidal activity against *M. alternatus*. RNA expression profiles of the midgut proteases from different larvae instars of *M. alternatus* were analyzed by next generation sequencing. Based on our sequencing data, trypsin and chymotrypsin were selected, which are the major proteases that hydrolyze Cry pro-toxins and activate them in lepidoptera and diptera larvae. In addition, the potential cleavage sites from the candidate proteases were predicted and those sites were inserted into the loop domain I region of Cry3Aa to get a modified Cry3Aa. Our results show that the Cry3Aa modified toxin has higher toxicity against *M. alternatus* larvae compared with the original Cry3Aa toxin. This work will contribute to better understand the proteolytic processing barrier for Cry3Aa in *M. alternatus* midgut, as well as to provide a novel approach for the biological control of *M. alternatus*.

Contributed paper. Monday, 17:45, **57-STU**

Spanish strains of *Bacillus thuringiensis* as biological control agents against *Lobesia botrana* (Lepidoptera:*Tortricidae*) larvae

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The grape moth *Lobesia botrana* is the most destructive insect pest of vineyards in European, North African and Asian countries, and has recently been reported in America. This lepidopteran causes significant damage to grape production, leading to important economic losses. Chemical control has been effective for control of this pest, but recently pheromone mass trapping and bioinsecticides based on *Bacillus thuringiensis* (Bt) have been used successfully. Bt has a wide genetic diversity but the active ingredient of current commercial products is usually based on just a few Bt strains. The purpose of this study was to identify Spanish strains of Bt with higher toxicity than presently available Bt products, or with similar toxicity but harbouring different insecticidal genes, in order to preserve the inherent toxicity of Bt and its integrity as a biological control agent. We determined the insecticidal potential of two Spanish isolates, Leapi01 and Hu4.2, which had been previously characterized, in comparison with the commercial serovars *kurstaki* (DiPelo) and *aizawai* (Xentario) and several recombinant proteins (Vip3Aa45, Vip3Ag4 and Vip3Af3). Leapi01 showed the highest toxicity (1.67ng/cm2) for first instar *L. botrana* larvae. Interactions with Vip3Aa45 were also tested in order to determine possible synergistic effects to improve the biological control of this pest. The results of these studies will be reported.

Contributed paper. Monday, 18:00, **58**

New Insecticidal Proteins from Non-Bacillus thuringiensis Microbial Diversity

Lu Liu[†1], Jarred Oral[1], Dan Altier[2], Jessica O'rear[1], Barbara Rosen[1], James Le[1], Mark Mcdonald[1], David Cerf[1], Jon Robeson[2], Lisa Procyk[2], Adane Kassa[2], Weiping Xie[1], Genhai Zhu[1], Jennifer Barry[2], Claudia Pérez-Ortega[2], Nuria Jiménez-Juárez[2], Miles Cowart[2], Jian-Zhou Zhao[2], Ute Schellenberger[1], Nasser Yalpani[2], Jun-Zhi Wei[1], Virginia Crane[2], Gary Sandahl[2], Mark Nelson[2], Albert Lu[2], Gusui Wu[2]

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The soil bacterium *Bacillus thuringiensis* (Bt) has been a valuable source of insecticidal protein toxins useful for pest control of crop plants. Genes encoding the toxins have been used for developing insect control traits in transgenic crops. In fact, current commercial traits are all developed with insecticidal proteins from Bt strains. Here, we describe a strategic approach for new insecticidal protein discovery that explores non-Bt microbial diversity. We will present several examples of insecticidal proteins discovered from non-Bt microorganisms that have potent insecticidal activities. The potential of these non-Bt insecticidal proteins were examined and their mode-of-actions were compared to Bt toxins. Our results suggest that non-Bt microbial diversity is a rich source of new insecticidal proteins and some of those new protein toxins may be used for the development of new traits in crop plants for insect pest control.

Contributed paper. Monday, 18:15, **59**

Managing the evolution of resistance to biopesticides with genetically modified insects

Raymond Ben

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Genetically modified insects are of increasing interest as tools for managing pests. Supressible dominant lethal constructs, such as the RIDL techology developed by Oxitec, can be thought of as genetic replacements of sterile insect release. In both SIT and RIDL large numbers of released males supress the reproductive potential of females. However, if RIDL lethality is restricted to females it is possible both to suppress populations and to introgress substantial genetic material into pests via the male line. Earlier experiments and theory indicated that female specific RIDL can supress the evolution of resistance to *Bacillus thuringiensis* toxins. Using a range of selection experiments with Cry1Ac resistant diamondback moth, *Plutella xylostella*, we explored the conditions that would facilitate resistance management using both RIDL and a high dose / refuge strategy. We found that, compared to theoretical predictions, a relatively high release ratio and a low initial frequency of resistance alleles made resistance management with RIDL feasible. At moderate frequencies of resistance RIDL could significantly slow the evolution of resistance, but not avert it. At the lowest initial levels of resistance RIDL there was some evidence that RIDL could help stabilize resistance. Discrepancies between theory and experiments could be explained by the lower than expected competitivenes of RIDL males and by population dynamic effects. However, results overall suggest that RIDLs best use in resistance managements might be as a tool for targeted suppression of local resistant populations rather than via introgression.

TUESDAY – 26 July

SPECIAL SYMPOSIUM — Tuesday, 08:00-10:00 - *Vouvray*

Human impact on pathogens-honeybee interactions - *Aurore Dubuffet & Philippe Gayral*

SYMPOSIUM. Tuesday, 08:00 **60**

EPILOBEE: Results from a pan-European epidemiological study on honeybee colony losses 2012-2014, conducted by the European Union Reference Laboratory

Marie-Pierre Chauzat, Marion Laurent, Antoine Jacques, Epilobee Consortium, Laura Cauquil, Marie-Pierre Riviere, Mathilde Saussac, Stéphanie Bougeard, Pascal Hendrikx, Magali Chabert†

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For the first time, a harmonised active epidemiological surveillance programme on honeybee colony (*Apis mellifera* L.) mortality (EPILOBEE) was set up in 17 European Member States for two years. The national protocols were based on guidelines issued from the European Union Reference Laboratory for Honey Bee Health (EURL). Three visits were performed by bee inspectors: before winter, after winter and during the beekeeping season. Farming practices and clinical manifestations of the main infectious and parasitic diseases were recorded through a detailed questionnaire. The objective of the two-year programme was to get a state of play of honeybee colony losses on a harmonized basis in each of the participating Member States. Simultaneously, the main honeybee diseases were investigated based on case definitions and sampling protocols provided by the EURL. Winter colony mortality rates ranged from 3.2% to 32.4% and from 2.4% to 15.4% during the first year and the second year of the programme, respectively. This programme was a descriptive epidemiological study enabling the collection of official and comparable data on honey bee health during two years with a methodology that was fully feasible and repeatable. Data gathered throughout these two years on various topics were statistically analysed, to explore correlations between the colony losses and some risk factors. The outcomes of EPILOBEE were an essential prerequisite to the implementation of future explanatory studies investigating the potential causes of honeybee colony losses such as pesticides and their possible interactions with pathogens.

SYMPOSIUM. Tuesday, 08:30 **61**

Honey bee stressor interactions: never the same, and so much to learn

Geoffrey Williams

University of Bern and Agroscope, Switzerland

Movement of honey bees and their products within and among continents has led to global homogenization of their parasites. Throughout the northern hemisphere, honey bee colonies are concurrently parasitized by recent invasive species that include among others, the fungus *Nosema ceranae* and the mite *Varroa destructor*. Additionally, colonies employed for pollination services in agro-ecosystems frequently face exposure to agricultural chemicals such as insecticides and fungicides; this may incite increased susceptibility to parasitism. The neonicotinoid class of systemic insecticides has received incredible attention of late. Laboratory and field studies employing individual honey bee workers demonstrate both lethal and sub-lethal effects; however, relatively few data exist concerning interactions between these chemicals and invasive parasites. Here I will provide a state of the art overview of simultaneous neonicotinoid-parasite pressures in honey bees, and discuss future avenues of research that will be most relevant to improving our understanding of increased honey bee colony mortality that is observed in many regions of the world.

SYMPOSIUM. **62**
Cancelled

SYMPOSIUM. **63**
Cancelled

SYMPOSIUM. Tuesday, 09:15 **64**

Man-made epidemics: Varroa and DWV in honeybees and the risk they pose to wild pollinators

Lena Wilfert[1], Robyn Manley[†1], Mike Boots[2]

1 Centre for Ecology and Conservation, University of Exeter, United Kingdom; 2 Integrative Biology, UC Berkeley, United States

Honeybees are arguably one of the most intensively managed insect species, while still free to interact with other wild pollinators, sharing both floral resources and pathogens. Anthropogenic influence can drastically alter the epidemiology of host-parasite interactions in this system. Deformed Wing Virus is a particular case in point: this virus has long been associated with honeybees, but was considered largely benign. In combination with *Varroa desctructor* however – an emerging ectoparasitc mite that can directly transmit the virus to the bee's hemolymph, circumventing many of its anti-viral defence mechanisms – this virus is associated with a risk in over winter mortality of honeybee hives. We have shown that Deformed Wing Virus is a re-emerging disease in honeybees, its current global epidemic fuelled by Varroa but driven by European populations of *Apis mellifera*. This virus is however not limited to honeybees, and we will discuss our current work on the risks this poses to managed and wild pollinator communities.

SYMPOSIUM. Tuesday, 08:00 **65**

Entomopathogenic fungi: Friend or enemy of the plant?

Rob Van Tol†[1], Gerrie Wiegers[1], Marilena Palmisano[2], Jurg Grunder2
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More than a decade ago it was discovered that belowground natural enemies of insects use alerting semiochemicals emitted by the attacked host plant to find their host. We hypothesized that non-mobile natural enemies, such as entomopathogenic fungi, must have developed similar strategies to increase their chances in contacting potential hosts. First reports of weevil larvae attracted to roots inoculated by such a fungus indicate that fungi may exploit the plant. Recently it was shown that specific fungal species and strains of *Metarhizium* and *Beauveria* are rhizosphere competent and closely associated with specific plant species. These different phylogenetic groups of fungal entomopathogens may display different strategies in their association with the specific plant species. We studied this system in soil for grubs of the cockchafer *Melolontha melolontha*. It appears that these fungi are very common in the rhizosphere of plants in more extensively managed meadows and that the interaction of the plant-fungus has influence on the pest density in these fields. Olfactometer trials indicate that some plant-fungus interactions attract the grubs while others do not. Unclear is what semiochemicals are involved in this attraction. Are the fungi or the plants signalling? Are the fungi manipulating the plants to attract the grubs? The fungus benefits from the attraction but for the plant it seems fatal. On ecosystem level the plant species may however profit from the sacrifice of individual plants. How has selection pressure favoured this interaction if the benefits for the individual plant are absent? Is the plant-fungus-insect interaction shaping the plant community? Several of these issues and hypotheses on this relation will be discussed.

SYMPOSIUM. Tuesday, 08:30 **66**

Potential of root-associated pseudomonads with insecticidal activity for biological control of soil-dwelling insect pests of crops

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Pest insects cause significant yield and quality losses to agricultural crops every year and those attacking the belowground parts of plants are particularly difficult to control. *Pseudomonas* bacteria are commonly associated with plants and thus are likely to be ingested by plant-feeding insects. Some pseudomonads, notably members of the *Pseudomonas protegens* and *Pseudomonas chlororaphis* groups, have developed strategies to protect themselves against the immune defenses of insects. These excellent root colonizers exhibit potent insecticidal activities. They are capable of killing or otherwise hampering the development of certain plant pest insects and of exploiting them as an alternative source of nutrients. Within the frame of the Swiss National Research Programme NRP68 on the sustainable use of soil as a resource, we investigate the potential of the entomopathogenic pseudomonads as biological control agents of soil-dwelling insect pests in greenhouse and field experiments. Targeted pests include larvae of root flies on vegetable crops and *Diabrotica* beetles on corn. An emphasis of our approach is on the application of mixtures of biocontrol agents, in particular on combinations with entomopathogenic nematodes, to take advantage of the distinct insect infection strategies deployed by these microorganisms. Application methods envisage the embedment of the entomopathogenic microorganisms in polymer capsules to improve their biocontrol efficacy and their shelf life.

SYMPOSIUM. Tuesday, 09:00 **67**

Threesome in the rhizosphere: bacteria, entomopathogenic nematode, and plant interactions

Ivan Hiltpold[1], Michael Brownbridge[2]
1 Western Sydney University (WSU), Australia; 2 Vineland Research and Innovation Centre Vineland Station, Canada

The rhizosphere, this region directly influenced by roots and root exudates, is a very active niche where several taxa coexist. As the primary source of carbon and nitrogen, roots play a pivotal role in this ecosystem where finely balanced interactions have evolved. Up to 20% of the photosynthesized carbon is exuded via roots in the rhizosphere, often shaping this environment to favor beneficial communities and hinder detrimental organisms. In return, rhizospheric organism, such as bacteria, favor nutrient mobilization or root growth via exudate too. It is well documented that volatile organic compounds (VOCs) emitted by bacteria are influencing root growth and branching or plant resistance to insect herbivores or pathogens. It has recently been shown that rhizospheric bacteria VOCs also impact above ground third trophic level. Indeed insect damage plants emit VOCs attracting parasitoid wasps toward the pests. On maize, bacteria VOCs may interfere in these tritrophic interactions, rendering the exploitation of parasitoids as bio-control agent difficult. Not only leaves, but also insect damaged roots emit VOCs to attract beneficial organisms. In the rhizosphere, entomopathogenic nematodes are attracted toward insect damaged root systems, following plumes of VOCs, enhancing the biological control of the targeted pest. Such interactions have been described in several systems and potentially deeply impact soil-dwelling insect pest management.

SYMPOSIUM. Tuesday, 09:30 **68**

Entomopathogenic Nematodes Boost Plant Immunity

Parwinder Grewal
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Entomopathogenic nematodes (EPNs) *Steinernema* and *Heterorhabditis* serve as important biological control agents for soil-inhabiting insect pests in many high-value cropping systems. EPNs have also been shown to be antagonistic to plant-parasitic nematodes but the

mechanisms are poorly understood. We discovered that the soil application of EPNs can induce components of systemic resistance in treated hosta and *Arabidopsis* plants. Here we hypothesized that EPN- induced systemic resistance is of broad spectrum with activity against chewing insects, sucking insects, and bacterial pathogens. We tested this hypothesis by comparing the development of a generalist chewing pest, beet armyworm (*Spodoptera exigua*), a generalist sucking pest, sweet potato whitefly (*Bemisia tabaci*), and a bacterial pathogen *Pseudomonas syringae* pv tomato on EPN-treated and control tomato plants. *Steinernema carpocapsae* -infected waxworm (*Galleria mellonella*) cadavers were applied to the soil around tomato plants in pots whereas the control plants received freeze-killed waxworms. EPN-induced defense responses were evaluated at 3, 7 and 15 days after treatment (DAT). We observed that the soil EPN-treatment had significant negative impact on all three organisms on tomato leaves 3 or 7 DAT, but not 15 DAT. Treatment with EPNs delayed immature beet armyworms from reaching the next developmental stage, impaired whitefly egg hatch, and reduced lesion formation of the bacterial pathogen on the leaves. While the evolutionary significance of this phenomenon is not yet understood, the findings suggest that soil applications of EPNs can provide benefits beyond the target insect control by boosting general plant immunity.

CONTRIBUTED PAPERS — Monday, 08:00-10:00 - ***Courteline***

Bacteria 2 - *Marianne Carey & Shuyuan Guo*

Contributed paper. Tuesday, 08:00, **69**

Cry1Ac toxin mode of action in heliothines

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The Cry1Ac protein produced by the bacterium *Bacillus thuringiensis* var. *kurstaki* HD-73 is synthesized in the form of a 120 kDa protoxin form that is processed in the alkaline pH of the lepidopteran midgut by proteases to a 60 kDa toxin core that specifically binds to receptor(s) on the brush border membrane of enterocytes. Larvae of taxonomically-close *Heliothis virescens* and *Helicoverpa zea* are considered susceptible to Cry1Ac, yet their relative susceptibility to the toxin differs > 60-fold. Since Cry1Ac binds with high affinity to similar receptor proteins on midgut cells from both species, we hypothesized that the difference in susceptibility to Cry1Ac is related to differences in the processing of the protein in the midgut. In this study, we tested this hypothesis by comparing proteolytic processing of Cry1Ac protoxin by gut fluids from larvae of both species. We report differences in protoxin processing that may explain differences in relative susceptibility to Cry1Ac among these heliothine species.

Contributed paper. Tuesday, 08:15, **70**

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

Julia Bally[1], Glen Mcintyre[2], Rachel Doran[1], Ignacio Larrinua[3], Kenneth Narva[3], Peter Waterhouse[1,2]
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Helicoverpa armigera, the cotton bollworm, is a major insect pest for a wide range of agricultural crops. It causes huge yield losses not only through feeding damage but also by increasing the crop's vulnerability to bacterial and fungal infection. *H. armigera* has evolved substantial resistance to most of the available classes of chemical insecticides, prompting the development of transgenic crop plants with alternative insect-resistance-conferring mechanisms. For example, transgenic crops producing Bacillus thuringiensis (Bt) toxins have been very successful. However, there is still a concern about insect populations emerging with resistance to such biopesticides. Novel strategies that give protection as effective as conventional insecticides, without affecting the environment, need to be continuously developed and improved. Trans-kingdom RNA interference, when double-stranded (ds) or hairpin (hp) RNA are expressed in transgenic plants to silence essential genes within herbivorous pests, has emerged as a promising strategy for managing devastating crop pests. However, the dicing of duplexed RNA into siRNAs by the plant RNAi machinery may reduce the pesticidal activity. In our study, we introduced a hairpin RNA construct, targeting the acetylcholinesterase (ACE) gene of *Helicoverpa armigera*, into the chloroplast genome of *Nicotiana benthamiana*. As the chloroplast lacks RNA interference machinery, the hpRNAs were not processed into siRNAs in the transplastomic lines, and the plants were protected against *H. armigera* herbivory. This was correlated with inhibition of larval growth and reduction in ACE activity. These findings suggest that chloroplast expression of hpRNA can provide durable pest resistance in crops.

Contributed paper. Tuesday, 08:30, **71**

Lysinibacillus sphaericus Binary toxin structure revealed *in situ* by *de novo* phasing with an X-ray free-electron laser: Insights into the larvicidal biology of BinA and BinB

Jacques-Philippe Colletier[1], Michael Sawaya[2], Jose Rodriguez[2], Duilio Cascio[2], Dennis Bideshi[3], Robert Hice[3], Brian Federici[†4], David Eisenberg[2]
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The *L. sphaericus* Binary protein is a crystal larvicide effective against many mosquitoes, including malaria vectors of the *Anopheles gambiae* species complex, and most *Culex* species, important West Nile virus and filarial worm vectors. The binary (Bin) crystals contain two homologous molecules, BinA and BinB that play distinct roles in the multi-step intoxication process. Structural studies of Bin have been impeded by their small size. As a result, after decades of research the mechanisms that trigger progression from harmless, robust crystals, to soluble protoxin heterodimers, to internalized mature toxin, remain poorly understood. Using large Bin crystals synthesized through genetic engineering, we solved the BinAB structure using serial-femtosecond crystallography (SFX) at an X-ray free-electron laser (XFEL) source. Using three heavy atom derivatives, we show the feasibility of *de novo* SFX phasing with a crystallographic asymmetric unit nearly three fold larger than previously phased *de novo* by SF. The structure reveals tyrosine and carboxylate-mediated contacts as pH sensitive switches that solubilize protoxin in alkaline larval midguts. An enormous heterodimeric interface appears responsible for anchoring BinA to receptor-bound BinB for co-internalization. Remarkably, this interface is largely composed of propeptides, suggesting proteolytic maturation would

trigger heterodimer dissociation and progression to cell internalization and possible pore formation. The structure offers rational means to test hypotheses about intoxication pathways and perhaps for engineering broader target spectra that would include *Aedes* species like *Ae. aegypti*, an important vector of Dengue Fever and Zika viruses.

Contributed paper. Tuesday, 08:45, **72**

A binB knockout in *Lysinibacillus sphaericus* demonstrates BinA can form a crystal without BinB in *Bacillus thuringiensis*

Hyun-Woo Park[1], Dennis Bideshi[1], Brian Federici[2]

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The Bin toxin is a mosquitocidal crystal protein produced by certain strains of *Lysinibacillus sphaericus* during sporulation. It is composed of the 42-kDa toxic domain and the 51-kDa binding domain, and the genes encoding these proteins are in an operon. *L. sphaericus* 2362 produces the most toxic Bin although the size of the crystal is much smaller than endospore. To improve the toxicity and yield of *L. sphaericus* 2362 Bin, we previously constructed a recombinant *Bacillus thuringiensis* subsp. *israelensis* 4Q7 strain that contains pPHSP-1, a plasmid harboring the *bin* operon under control of *cyt1A*-p/STAB-SD expression system. Bin crystals produced by this recombinant are about eight-fold larger than those produced by the wild-type *L. sphaericus* 2362. However, it remains controversial whether BinA or BinB can (1) form a crystal independently and (2) have toxicity alone to target mosquito larvae. Therefore, we knocked-out *binA* or *binB* on pPHSP-1 using unique restriction enzymes, self-ligated each, respectively named pPHSP-ΔbinA and pPHSP-ΔbinB, and synthesized each protein in the above acrystalliferous strain of *Bacillus thuringiensis* subsp. *israelensis*. The results show that 4Q7/pPHSP-Δ*binA* in which *binB* is intact does not form a crystal whereas 4Q7/ pPHSP-ΔbinB in which *binA* is intact forms a crystal. SDS-PAGE and mosquito larval bioassay were performed to determine the toxicity against *Culex quinquefasciatus* 4th instars.

Contributed paper. Tuesday, 09:00, **73**

Structure and activity of the Cry6Aa pesticidal toxin

Colin Berry, Alexey Dementiev, Jason Board, Anand Sitaram, Timothy Hey, Matthew Kelker, Xiaoping Xu, Yan Hu, Cristian Vidal-Quist, Vimbai Chikwana, Samantha Griffin, David Mccaskill, Nick Wang, Shao-Ching Hung, Michael Chan, Marianne Lee, Jessica Hughes, Alice Wegener, Raffi Aroian, Kenneth Narva

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The Cry6 protein of *Bacillus thuringiensis* is a powerful toxin with potential for use against coleopteran and nematode pests of agriculture. The proteins are unrelated to other invertebrate- active toxins (including other Cry proteins) at the primary sequence level and our understanding of their structure and function has been poor, thus limiting our ability to manipulate the toxins to modulate their activity. We have now solved the structures of the proform and trypsin- activated Cry6Aa protein to 2.7 and 1.8 Å respectively and demonstrated a pore forming mode of action. The toxins share structural homology to highly alpha helical toxins such as haemolysin E from *Escherichia coli*, which is consistent with pore formation mediated through a surface loop and mutation of the equivalent loop in Cry6Aa abolishes activity. We conclude that Cry6 proteins act in a manner distinct from other invertebrate-active toxins and further studies of its structure and function are now possible.

Contributed paper. Tuesday, 09:15, **74**

The *Bacillus thuringiensis* toxin Cry6Aa1 forms ionic channels in giant liposomes

Vincent Vachon[1], Maxime Schmidt[1], Timothy Hey[2], Xiaoping Xu[2], Samantha Griffin[2], Vimbai Chikwana[2], David Mccaskill[2], Ken Narva[2], Jean-Louis Schwartz[1,3]

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The mechanism of action of the nematocidal and insecticidal *Bacillus thuringiensis* toxin, Cry6Aa1, was studied by monitoring its ability to release the fluorescent dye carboxyfluorescein from giant liposomes composed of phosphatidylcholine and phosphatidylglycerol in a 9:1 ratio. In comparison with liposomes prepared using a wide variety of techniques, such giant liposomes greatly facilitated the detection of toxin activity. Because carboxyfluorescein molecules carry two or three negative charges at near neutral pH, their efflux from the liposomes necessitates either the concomitant efflux of cations or influx of anions. Experiments carried out under various ionic conditions indicate that the toxin forms pores which allow such ionic fluxes across the membrane and exclude the possibility that it acts by destabilizing the membrane or by causing osmotic lysis of the liposomes due a massive influx of solutes. These results also confirm the previous observation, from planar lipid bilayer studies, that the native form of Cry6Aa1 can permeabilise membranes without the need for proteolytic activation. Nevertheless, different preparations obtained by chromatography following incubation of Cry6Aa1 with midgut juice extracts from the coleopteran species *Diabrotica virgifera virgifera* were significantly more active than the untreated toxin (Supported by an NSERC CRD Grant in partnership with Dow AgroSciences Canada Inc.).

Contributed paper. Tuesday, 09:30, **75-STU**

Two polysaccharides are involved in the formation of specific biofilm structures in *B. thuringiensis*

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Biofilms produced by *B. thuringiensis* in glass tubes include a pellicle floating on the culture medium and a ring, which sticks to the tube walls and circles the pellicle. We searched for the *B. thuringiensis* genetic determinants involved in the biosynthesis of the polysaccharidic component of the biofilm matrix, and deleted a large chromosomal locus, which we named *eps1*. The *eps1* locus includes genes encoding enzymes and

transporters predicted to be involved in exopolysaccharides biosynthesis. However, deletion of *eps1* resulted only in the suppression of the biofilm ring, and the *eps1* mutant biofilm appeared as a pellicle floating freely on the culture medium. We subsequently found that *eps1* is involved in the production of a capsule, which is produced in low oxygen concentration and is extremely adherent on biotic and abiotic surfaces. We hypothesized that a second genetic determinant should be involved in the biosynthesis of the exopolysaccharides required for the production of the biofilm pellicle. We identified a chromosomal locus smaller than *eps1* and displaying all the elements required for exopolysaccharides biosynthesis. The deletion of this locus, which we named *eps2*, led to a biofilm, which included a ring but no pellicle. We also determined that *eps2* was not required for adhesion on biotic or abiotic surfaces. Therefore, *B. thuringiensis* uses a capsular polysaccharide to build the biofilm ring, and an exopolysaccharide to build the biofilm pellicle. The two polysaccharides are required to give a complete biofilm since a co-culture of the *eps1* and *eps2* mutants grown in 1:1 ratio restores the full biofilm. This work shows for the first time that different *eps* loci are involved in the formation of specific structures of the biofilm.

Contributed paper. Tuesday, 09:45, **76**

Enzymatic activity of *Bacillus thuringiensis* toxins

David Pauron†, Marcel Amichot, Marie-Paule Esposito, Armel Gallet
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When sporulating, *Bacillus thuringiensis* produces paracrystalline inclusions containing one or more proteins which are mainly responsible for the entomopathogenic properties of this bacterium. Such proteins mostly belong to the Cry family which, after spore lysis and solubilization of the crystals, are delivered to specific zones of the gut. Then, they bind to (a) receptor(s) to induce final lysis of intestinal epithelial cells. The potency of these toxins has been linked to the hydrolysis of inactive bigger protoxins. Such an activation is thought to occur in the digestive tract before the toxins reach their final destination. Moreover, several reports indicate that some of the Cry toxins undergo a superactivation step which allows them to multimerize and form ionic pores throughout the plasmic membranes of enterocytes. Until now the catalytic process allowing this superactivation remains unclear. We now report that some Cry toxins display a protease activity when assayed in adequate conditions. Interestingly, among these toxins are those believed to superactivate. Analysis of reported 3D structures of these toxins allowed us to pinpoint amino-acids putatively involved in the catalytic process. When these amino-acids are mutated, the activity of the resulting toxins is altered which confirms that they are indeed involved in the process. Surprisingly, we have been able to detect such an activity in other toxins which do not belong to the Cry family but which also have entomopathogenic properties. Hence such a property may be shared by much of *Bacillus* toxins which may constitute a milestone in the entomopathogenic power of such bacteria.

CONTRIBUTED PAPERS — Monday, 08:00-10:00 - ***Bourgueil***

Fungi 1 - *Ann Hajek & Helen Hesketh*

Contributed paper. Tuesday, 08:00, **77**

Entomophaga maimaiga in _Lymantria dispar_ in Eastern Europe

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Entomophaga maimaiga is native to Japan, northeastern China and the Russian Far East where it kills gypsy moth, Lymantria dispar, larvae. Gypsy moth was introduced to North America in 1869 and 120 years later *Entomophaga maimaiga* was first found in 7 northeastern states. Although gypsy moth is native to temperate Europe, this fungus was not previously reported from Eastern Europe. In 1999, the fungus was successfully introduced to Bulgaria where it was distributed throughout Bulgarian oak forests. Between 2011 and 2013, *E. maimaiga* spread incrementally, reaching Slovakia to the north. Based on studies in the United States, we assume that spread was primarily due to long distance windborne movement of conidia. Studies of non-target effects in Slovakia have yielded no evidence of non-target effects.

Contributed paper. Tuesday, 08:15, **78**

Termite choice of direction to pathogen odor related with nestmate olfactory signals

Aya Yanagawa[1], Tomoya Imai[1], Toshiharu Akino[2], Toshimitsu Hata[1], Tsuyoshi Yoshimura[1], Fumio Yokohari[3], Yoshihiro Toh[4]
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Termites tend to avoid the pathogen-odor direction in the Y-maze test, but approach and groom their infected nestmates to remove the pathogens such as a fungal conidium from their nest mate cuticle to maintain healthy population. To clarify their sensitivity to pathogen- related odor signals and the association of the signals in termite hygiene behavior, we tested termite olfactory perception by behavioral induction and choice of the direction in Y-maze test. The association in odor signals and choice of the direction was also examined in Y-maze test by mixing the aversive odor from the entomopathogenic fungi *Isaria fumosorosea* with other signals such as odors from nestmates or filter paper in the Y-maze test. As a result, when confronted with a choice between branches with/without fungus odor, termites tended to avoid the fungus odor branch, and termites chose the branch that contained their nestmate odor mixed with the fungus odor only when they could not choose a branch without fungus odor. Then, previously, since it was indicated that termite used the signals received by antennae to perceive the fungal odor, sensilla on termite antenna were observed by SEM and TEM.

Contributed paper. Tuesday, 08:30, **79-STU**

Natural occurrence of *Beauveria bassiana* in soil, as infections in stink bugs and as endophytes in bean plants, from organic and conventional fields in Cuba

Yordanys Ramos[1], Orelvis Portal[2], Erik Lysøe[3], Annarella Chea[1], Luis Rojas[4], Nicolai Meyling[5], Ingeborg Klingen[†3]
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The aim of this study was to evaluate the effect of conventional versus organic common bean (*Phaseolus vulgaris*) production on natural occurrence of *Beauveria* spp. as entophytes in bean plant tissue, from soil and as infections in stink bugs (Hemiptera: Pentatomidae), an important pest of bean in Cuba. Twenty-four organic and conventionally managed bean fields were sampled from September 2014 to April 2015 and *Beauveria* spp. were isolated and DNA extracted. PCR amplification of the intergenic Bloc region was performed for the identification of *Beauveria* species. Eighty-seven isolates were obtained from the soil samples by using the *Galleria mellonella* baiting technique. Further, 45 isolates were obtained from endophytic tissues of bean plant parts and 18 isolates were acquired from stink bugs. Only *Beauveria bassiana* was identified by DNA sequencing in this material. *B. bassiana* was more prevalent in soil, plant and stink bugs sampled from organic fields (41% soil, 22% plant, 9% bugs) compared to conventional fields (17% soil, 8% plant, 2% bugs). All plant parts were colonized by *B. bassiana*, but a significantly higher occurrence of this fungus was found in roots (9%) compared to stems (6%), leaves (4%) and pods (2%) in organic fields. In conventional fields there was a significantly higher occurrence of *B. bassiana* acquired from root (4%) and stem (3%) compared to leaves (1%) and pods (1%). Mating type PCR assays revealed that each of the isolates carried single mating types, with frequencies of 146/150 (MAT1) and 4/150 (MAT2), indicating limited potential for recombination. Our findings show that *B. bassiana* occur naturally as endophytes in bean fields in Cuba and contribute to a better ecological understanding of *B. bassiana* in agriculture.

Contributed paper. Tuesday, 08:45, **80-STU**

Biotic and abiotic factors influencing the virulence of Entomophthoromycota on aphids in cereals

Stéphanie Saussure[†1], Cecilie Sletteng[2], Nina Trandem[1,2], Karin Westrum[1], Ingeborg Klingen[1]
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Aphids in cereals are an important problem in Europe. Entomopathogenic fungi in the Phylum Entomophthoromycota are among their natural enemies. Under certain conditions, they can cause epizootic events and control pest aphid populations. This epizootic development is affected by many abiotic and biotic factors such as aphid species and their host plant (including weeds within the crop), fungal species and isolates, and temperature. Studies from Denmark, UK, Slovakia and suggest that the genus *Pandora* is the most prevalent fungal pathogen of the English grain aphid (Sitobion avenae). Which fungal species that is the most prevalent in populations of the other important aphid species in cereals in Europe, the Bird cherry-oat aphid (*Rhopalosiphum padi*), is less clear. We chose, however, to use Pandora to assess the biological control potential of Entomophthoromycota against aphids in cereals and to produce data that might be used in a pest-warning model incorporating the effect of this natural enemy. This was done by conducting laboratory studies on the virulence of two *Pandora* isolates (collected in the same field) on *R. padi* and *Myzus persicae* at three temperatures (12, 15 and 18◦C). *M. persicae* is a polyphagous aphid that may be present on weeds. It can be an alternative host for *Pandora* and hence might also affect the epidemic development of *Pandora* in aphids that are cereal pests. Our preliminary results show that *R. padi* is more resistant to the tested *Pandora* isolates than *M. persicae*. The two *Pandora* isolates had different virulence in the two aphid species tested. The temperature did not influence the virulence.

Contributed paper. Tuesday, 09:00, **81**

Entomophthoromycota pathogens of insects from Argentina. An updated review

Claudia Lopez Lastra, Romina Manfrino, Alejandra Gutierrez
Centro de estudios parasitologicos y de vectores, Argentina

Entomophthoralean fungi had been poorly studied in Argentina until about 2000 there had been very few reports of insects infected with Entomopthorales. Agricultural insect pests had been mostly surveyed from horticultural, soybean, rice and cereal crops and several species were identified infecting insect hosts. Some few of the species were isolated in axenic pure cultures and deposited at Culture Collections. Up to present time nine species of Entomopthoralean fungi had been recorded in agricultural pests and also from sanitary insect vectors as houseflies. A total of nine fungal species and several strains and an abroad list of insect hosts had been surveyed from pampasic and Litoral region of Argentina including Buenos Aires, Santa Fe, Entre Rıos, and Corrientes provinces. The orders of insect hosts that were recorded were: Lepidoptera, Hemiptera, Thysanoptera, and Diptera. The morphological features were studied as also type and nuclei number, diameters of conidia and nuclei, rizoids, cystidia presence and resistance spores. For some of the species also molecular characterization was done to confirm ID. The list of fungal species recorded *Entomopohthora planchoniana* Cornu, *E. ferdinandii*, S. Keller *Pandora neoaphidis* (Remaudiere & Henebert) Humber, *Zoophthora radicans* (Berf.) A. Batko, *Zoophthora* sp, *P. neoaphidis*, *Pandora* sp. *Pandora dipterigena* (Thaxt.) Humber *Conidiobolus obscurus* (I.M: Hall & P.H. Dunn) Remaud. & S. Keller *C. coronatus* (Costantin) *Batko* and *Batkoa* sp. *Neozygites. fresenii* (Nowak.) Remaud. & S. Keller and *Neozygites* sp. were deposited at Fungal Culture Collection and at Fungarium of CEPAVE, La Plata and in ARSEF Culture Collection, Ithaca, NY., U. S. A.

Contributed paper. Tuesday, 09:15, **82-STU**

Incidence of fungal infections on Neotropical ants: environment or the host, which has more influence?

José Pablo Barrantes[†1], Maria José Monge-Salazar[1], Milagro Granados-Montero[2], Priscila Chaverri[1,3]
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Ants, like any other living organism, are prone to disease and are mostly caused by fungi of the order Hypocreales (Ascomycota). Due to the importance that ants and fungi have on ecosystems and human life, we evaluated the effects of some environmental variables in the incidence of fungal infections. Our goal was to determine whether this incidence of infections depends more on the characteristics of each ant species or on environmental factors. To attain this, we searched for anthills of *Atta cephalotes* and *Paraponera clavata* in a second-growth tropical rainforest in Costa Rica. When found, we systematically counted the number of infected bodies near the anthills. When all infected ants were collected, we measured environmental variables in each nest. In addition, we made laboratory experiments to test the resistance of four ant species against the fungi *Beauveria* and *Aspergillus*. In the field, we found that the incidence of infections does not vary between nest sites, but among the species infected with fungi; *P. clavata* was the most affected. Environmental variables did not differ between the two ant species (*A. cephalotes* & *P. clavata*) nest sites, and only the amount of organic matter in the soil had an effect on the incidence of infections. Finally, the species of ants with greater predatory habits were the most resistant to the artificial infections, followed by the omnivorous and lastly, the mycophagous and herbivorous. We conclude that the immunity of the evaluated ants against infections is mainly mediated by own defenses of the species, like specialized social habits or physiological conditions, and not by environmental factors. The findings in this study are relevant to conservation efforts and biological control purposes.

Contributed paper. Tuesday, 09:30, **83-STU**

Impacts of conventional and organic agriculture on soil-borne entomopathogenic fungi

Eric Clifton[1], Stefan Jaronski[2], Erin Hodgson[1], Aaron Gassmann[1]
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Entomopathogenic fungi kill agricultural pests and can diminish pest outbreaks. Species such as *Beauveria bassiana* and *Metarhizium anisopliae sensu lato* are significant mortality factors for several insects. A better understanding of these organisms is an essential step in developing strategies to conserve entomopathogenic fungi in agricultural systems. We hypothesize that 1) organic farming methods better suit populations of soil-borne entomopathogenic fungi, and 2) conventional farming methods, coupled with the use of synthetic chemical inputs, could have negative impacts on these beneficial fungi. In 2011 and 2012, soil samples were collected from organic and conventional fields of corn and soybean and the field margins. Entomopathogenic fungi in the soil samples were quantified with larval *Galleria mellonella* and colony forming units were counted on plates with selective growth medium. Field history and soil properties were analyzed with multiple regression to determine what factors may affect the abundance of soil- borne entomopathogens. In 2011, occurrence of *M. anisopliae* s.l. and *B. bassiana* was lowest in conventional fields. The average number of *M. anisopliae* s.l. colony forming units per gram of dry soil was lowest in conventional fields. Multiple regression analysis revealed that abundance of *M. anisopliae* s.l. was positively associated with applications of organic fertilizer and silt content, and negatively associated with nitrogen content, tillage, and herbicide applications. In 2012, there was no significant difference in the abundance and occurrence of entomopathogenic fungi among treatments.

Contributed paper. Tuesday, 09:45, **84**

Effect of a predatory mite on transmission of the fungus *Neozygites floridana* in *Tetranychus urticae* populations

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1 Norwegian University of Life Sciences, Norway; 2 J.K. Pell Consulting, United Kingdom; 3 Norwegian Institute of Bieconomy Research, Norway
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The two-spotted spider mite, *Tetranychus urticae*, is a serious pest of numerous crops worldwide. Sustainable management solutions for *T. urticae* include predators and entomopathogens. *Neozygites floridana* is a naturally occurring obligate fungal pathogen of *T. urticae* and can cause declines in *T. urticae* populations. The purpose of this study was to determine whether releasing the predatory mite *Phytoseiulus persimilis* into *T. urticae* populations has the potential to increase transmission of *N. floridana* and accelerate the development of an epizootic. This is the first study quantifying the effect of *P. persimilis* on transmission of *N. floridana* to *T. urticae* in a controlled microcosm study. Our results show that introducing *P. persimilis* into *T. urticae*, populations increased the proportion of *T. urticae* infected with *N. floridana*. By the final sampling occasion, the number of *T. urticae* in the treatment with both the predator and the pathogen had declined to zero in both experiments, while in the fungus-only treatment *T. urticae* populations still persisted. We suggest that releasing *P. persimilis* into crops in which *N. floridana* is naturally present has the potential to improve spider mite control more than through predation alone.

MICROBIAL CONTROL DIVISION SYMPOSIUM — Tuesday, 10:30-12:30 - ***Chinon***

Next Generation Biopesticides - *Carrie Hauxwell*

SYMPOSIUM. Tuesday, 10:35 **85**

Using bumblebees for targeted application of biopesticides

Sarah Van Beneden, Soraya Franca Marlies Vleugels, Felix Wäcker
Biobest Ilse Velden Westerlo, Belgium

For several years Biobest has been working on the development of their Flying Doctors system. This allowed us to provide the first commercial tool for insect vectoring of crop protection products. The pioneering system consists of a bumblebee hive with an integrated

product dispenser. Bumblebees leaving the hive, walk through the dispenser and become loaded with the biopesticide. By visiting the flowers during pollination, the biopesticides are being delivered in a targeted manner, making this technique highly suitable for control of flower associated diseases and pests. This application technique has several advantages over conventional spraying: due to targeted product delivery, far less product is required; it results in a continuous application and protection; and finally in considerable savings in labour. In several countries the Flying Doctors system is being used commercially to control *Botrytis cinerea* in strawberry and raspberry (including BE, NL, FR and FI). Prestop 4B, containing *Gliocladium catenulatum* J1146, is the first biopesticide in Europe being authorised for the application via bumblebees. *G. catenulatum* acts as a mycoparasite and competes in the flowers for nutrients and space, resulting in fewer latent *Botrytis* infections and significantly less fruit rot. Biocontrol agents suitable for this vectoring technique need to be selected carefully, fulfilling several criteria, such as effectiveness towards the target; compatibility with the vector; ability to colonize flowers. Formulation needs to allow optimal product adherence and dispersion. Biobest is investigating several new applications, including control of internal fruit rot in sweet pepper, fire blight in pear and apple; and thrips in several crops.

SYMPOSIUM. Tuesday, 11:00 **86**

Working with insect pathogen ecology for better biocontrol delivery

Michael Brownbridge[†1], Travis Glare[2]
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The demonstration that some insect pathogenic microbes, mainly fungi and bacteria, can have complex ecologies involving interactions in, on and around plants, provides new opportunities for their utilization and delivery. The common occurrence of entomopathogenic microbes as endophytes or displaying rhizosphere competence has exciting potential. Some endophyte groups are already exploited because they confer significant advantages to the plant in terms of improved performance in the face of abiotic and biotic stresses, including tolerance/resistance to pests and diseases. The ability to establish entomopathogens within a seed or seedling as a biocontrol agent avoids issues associated with the targeting of live organisms to a pest, particularly those with cryptic habits. Methods, which enable efficient targeted delivery and persistence of establishment will subsequently improve the degree of plant protection achieved. Delivery of entomopathogens via seed coatings to exploit their rhizosphere competence or endophytic ability is one method that is being investigated for both annual and perennial crops. Persistence and insecticidal activity of such approaches is currently under evaluation. But to fully utilize these associations to their best effect, an understanding of microbe-plant-environment interactions is essential. Tools to conduct such evaluations are now available, although not yet widely exploited for crop protection purposes.

SYMPOSIUM. Tuesday, 11:25 **87**

Characteristics of novel bacterial insecticides/miticides/nematicides from *Chromobacterium subtsugae* and *Burkholderia rinojensis*

Timothy Johnson, Pamela Marrone
Marrone Bio Innovations, United States

The novel bacteria *Chromobacterium subtsugae* strain PRAA-T1 and *Burkholderia rinojensis* strain A396 have been developed into effective insecticides, miticides and nematicides that contain no viable cells. The products known as Grandevo (*C. subtsugae*), Venerate (*B. rinojensis*) and Majestene (*B. rinojensis*) have received EPA registration with labels that include a broad variety of insect and mite pests and plant parasitic nematodes. Noteworthy activity has been demonstrated against certain Lepidoptera, Homoptera, Hemiptera, Coleoptera, and Diptera as well as a broad spectrum of plant damaging mites and parasitic nematodes. Bioactivity of both products is based on multiple metabolites produced during fermentation. The characteristics of both active ingredients and how they are successfully integrated into management programs via foliar sprays, soil applications and seed treatments will be presented.

SYMPOSIUM. Tuesday, 11:50 **88**

Regulatory implications of new technologies

Roma Gwynn
Rationale, United Kingdom

Bringing microbial control products to the market requires them to be regulated and if the intended use is for crop protection they need to be registered accordingly. Regulation for plant protection products have been developed over the last 50 years with a primary focus on conventional chemical pesticides. In recent years there has been work by organisations such as the Organisation for Economic Development (OECD) through their Biopesticide Steering Group (BPSG) to develop protocols and guidance that interprets the 'chemical' data requirements to make them applicable to microbial based products. The guidance and interpretation developed has principally considered the situation for conventional micro-organisms for example, *Beauveria bassiana, Metarhizium brunneum* where the spores are the active substance. Research is now focussing to develop microbial products that are innovative for example, these may have secondary compounds as the active principle, be endophytic or are from new micro-organism species. This presentation will consider the regulatory implications for innovative and new technologies, the implications for plant protection product regulation, how research links into this process and how it can support the development of new technologies.

Contributed paper. Tuesday, 10:30, **89-STU**

RACK1, a ribosomal protein involved in signaling, stress response and viral translation.

Evelyne Einhorn, Franck Martin, Carine Meignin, Jean-Luc Imler
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It is important to understand insect-virus interactions because (i) viruses impact beneficial pollinators, (ii) viruses can be used as control agents against harmful crop pests and (iii) arthropod-borne viruses become more and more prevalent. We use the model organism *Drosophila* to study host factors involved in antiviral immunity. RACK1 is a protein of the 40S ribosomal subunit, also associated with several signaling and adhesion molecules. We have identified RACK1 as a key factor for the translation of IRES-containing viral mRNAs. Moreover, RACK1 null mutants are unable to reach the adult stage, showing that RACK1 is required for development. Wild-type RACK1, but not a mutant impaired for the association with the ribosome, rescues development, indicating that RACK1 is involved in the selective translation of some endogenous mRNAs. Furthermore, in tissue culture cells RACK1 is required for the recovery from oxidative stress. Finally, signaling mutants of RACK1 can support translation of a viral IRES reporter in cells, indicating that the role of RACK1 in IRES-dependent translation is independent of its interaction with signaling cofactors. Interestingly, the so far ill-characterized "knob" region of RACK1 is required for IRES-dependent translation. Altogether, our *in vivo* and *ex vivo* data on RACK1 shed light on a factor that plays a critical role in the interaction with IRES-viruses at the interface of translation and cell signaling.

Contributed paper. Tuesday, 10:45, **90-STU**

Resistance to baculoviruses in the midgut of *Adoxophyes honmai*

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A strain of the smaller tea tortrix *Adoxophyes honmai* (Lepidoptera: Tortricidae) that is resistant to nucleopolyhedrovirus (NPV) was selected by exposing a field-collected *A. honmai* population to the 70% lethal concentration (LC70) of *Adoxophyes honmai* NPV (AdhoNPV) in the laboratory. The selected strain (Resistant strain; R-strain) showed over 67,000-fold higher resistance to AdhoNPV than the non-selected strain (Susceptible strain; S-strain). In addition, this R-strain has 850-fold cross-resistance to another baculovirus, *Adoxophyes orana* granulovirus (AdorGV). To reveal the mechanism of resistance to these two baculoviruses in R-strain larvae, the binding and fusion ability of occlusion-derived viruses (ODVs) was examined by fluorescence- dequenching assay. ODVs of AdhoNPV showed lower binding and fusion ability with midgut epithelial cells of R-strain than with those of S-strain, whereas ODVs of AdorGV showed no difference in binding or fusion with midgut epithelial cells of either R-strain or S-strain larvae. Viral gene expression in midgut epithelial cells and viral genome replication in whole larvae were examined by RT-PCR and quantitative PCR in R- and S-strain larvae. Following inoculation with AdhoNPV, viral gene expression and viral genome replication were undetectable in R- strain, although S-strain showed viral gene expression and viral genome replication. These results suggest that midgut-based resistance is critical for blocking AdhoNPV infection in larvae of R-strain. However, RT-PCR indicated that viral genes of AdorGV were expressed in midgut epithelial cells of R-strain. This suggests that the midgut resistance mechanism is not crucial for R-strain cross-resistance to AdorGV.

Contributed paper. Tuesday, 11:00, **91-STU**

Insect immune system to determine baculoviruses host specificity

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Baculoviruses are insect-specific DNA viruses with restricted host range, and serve as viral vectors for bioindustry applications such as foreign gene expression, vaccine production, and pest control. Autographa californica nucleopolyhedrovirus (AcMNPV), a prototype of a commercially available and widely used baculovirus, can infect 39 species in 13 families. Bombyx mori nucleopolyhedrovirus (BmNPV) is a major pathogen of silkworms and has developed high host specificity to *Bombyx mori*. Interestingly, on a genomic level, the AcMNPV and BmNPV are highly homologous, but they share no overlapping host range. These two quite similar viruses have extremely different infection outcomes in *Bombyx mori*. We theorize that the determination of host specificity may depend on virus-host interactions, and that several genes may be involved in determining host specificity. Therefore, we used next-generation sequencing (NGS) to analyze the transcriptome response of the hosts to these viruses. The transcriptome library was constructed, annotated, and grouped after sequence assembly. A comparison of gene expressions shows several significant differences in the gene expression profiles of BmNPV and AcMNPV, especially in cases where genes involved in immune responses are verified by RNA interference. The manipulation of virus-host specificity could provide a breakthrough for the application of baculovirus in protein expression systems and in the development of bio-control agents.

Contributed paper. Tuesday, 11:15, **92-STU**

The role of pathogen diversity on the evolution of resistance in an insect

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Microbial pesticides are an important component of organic and conventional horticulture and forestry. However, resistance against microbial pesticides has already occurred and is likely to become more prevalent with increased application. AcMNPV has recently been commercialized to control the cabbage looper, *Trichoplusia ni*. Wild-type AcMNPV contains many variants; however, specific strains are often selected for biocontrol

and the effect of that on the evolution of resistance is unknown. Lepidopterans can develop resistance to baculoviruses after multi-generational exposure in lab. We predicted that more diverse resistance mechanisms are likely involved in infections with multiple variants and individuals carrying resistant alleles for multiple variants are likely to be extremely rare; therefore, *T. ni* should develop resistance more slowly when treated with multiple variants of AcMNPV. AcMNPV variants were isolated using *in vivo* dilution cloning, characterized using RFLP and pathogenicity bioassays. A multi-generational experimental evolution experiment was carried out over five generations of selection using single and mixed (four variants and wild-type isolate) AcMNPV challenges. We found evidence of reduced resistance in diverse pathogen infections, as well as increased life history costs in diverse infections. Our findings provide insight into the role of pathogen diversity in the evolution of resistance, which could be applied to the development of more sustainable pest management strategies.

Contributed paper. Tuesday, 11:30, **93**

Pathogen competition in the cabbage looper, *Trichoplusia ni*: are multiple pathogens more effective than one?

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When multiple pathogens co-infect a host they may compete for host resources, have direct effects on each other or impact other pathogens indirectly via the immune response. These interactions could change the resulting mortality and speed of kill and, in the longer term, the evolutionary outcomes of the host-pathogen interaction. We understand little about how entomopathogens interact, nor whether applying multiple pathogens is likely to be beneficial for microbial pest control. To measure the impact of other pathogens on baculovirus success, we exposed the cabbage looper, *Trichoplusia ni* to TnSNPV followed by either the bacterium, *Bacillus thuringiensis*, or the fungus, *Beauvaria bassiana*. The fungus reduced the infection success of the baculovirus and the bacterium negatively impacted virus replication. This outcome has consequences for microbial control agents in insect pest management and adds to our fundamental understanding of the effect of mixed infections on pathogen and host populations.

Contributed paper. Tuesday, 11:45, **94**

Successive passages of mixed genotype virus populations: influence of the insect host colony

Benoit Graillot[1], Christine Blachere-Lopez[1,3], Samantha Besse[2], Myriam Siegwart[4], Miguel Lopez-Ferber[1]
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The development of field resistance to the virus isolate, CpGV-M, and the selection of CpGV populations able to control resistant insects offer a model to study the relationships between virus genotypes. Resistant insects provide a strong selection barrier for CpGV-M. Conversely, susceptible insects can be infected by both virus genotypes with quite similar efficacy. We have previously observed that CpGV-M is able to replicate in resistant insects when CpGV-R5 is present in a mixed virus population. Five mixed populations have been constructed by mixing Occlusion Bodies of CpGV-M and CpGV-R5 in various proportions. They have been replicated on susceptible or resistant insect populations. We have followed the pathogenicity (LC50,) both on susceptible and resistant insects for six successive passages. When the viral mixture is multiplied on the susceptible insects, the resulting viral isolates retain their effectiveness over time, whether on resistant or susceptible insects. On the other hand, the CL50s converge independently from the original frequency of each genotype when tested on the resistant insect colony. Higher variability is observed when challenging susceptible insects. When virus populations are on resistant insects, not such convergence of the LC50s is observed upon assays on susceptible or resistant insects.

Contributed paper. Tuesday, 12:00, **95**

Observations on a persistent baculovirus infection of *Trichoplusia ni* cells in culture

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Inoculation of *Trichoplusia ni* cells with an AcMNPV mutant lacking the p10 coding region, which was replaced by *beta-galactosidase* sequences, resulted in the survival of low numbers of cells that after three weeks appeared to be devoid of virus infection. These cells continued to multiply until they required dilution in fresh culture medium in new flasks. Further observations of these cultures revealed that some cells occasionally produced polyhedra, although with an apparent FP phenotype. Infectious budded virus titres from these cultures varied between 100 – 10000 pfu/ml. However, these virus stocks could be amplified in *Spodoptera frugiperda* cells to titres consistent with normal wild type virus infection. Further, when these stocks were used to inoculate *T. ni* cells they were unable to establish a persistent virus infection but instead killed all cells in the culture. Attempts to clone cells from the original persistent virus-infected cell population derived lines that always produced some budded virus. Occasionally, the persistent virus infection appeared to reactivate to an overt state with all cells producing occlusion bodies and eventually dying. Challenge of uninfected Hi5 cells with wild type AcMNPV resulted in a short term persistent virus infection for up to 10 sequential passages. Thereafter, all cells in the cultures succumbed to virus infection. Similar experiments with *S. frugiperda* cells failed to generate cultures with a persistent virus infection. The long term persistent virus infection observed with the AcMNPV p10 gene mutant in *T. ni* cells may be a consequence of the lack of cell lysis that is normally seen with this genotype. There may also be selection of a sub population of cells able to resist virus infection.

Molecular response of *Manduca sexta* immune tissues to parasitization by the bracovirus associated wasp *Cotesia congregata*

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Cotesia congregata is an endoparasitoid wasp that develops inside larvae of the tobacco hornworm *Manduca sexta*. This parasitoid wasp has evolved virulence strategies using an obligate viral symbiont named Cotesia congregata bracovirus (CcBV). CcBV particles, containing multiple double stranded DNA circles, produced by specialized cells of the wasp ovaries, are injected along with the eggs into the caterpillar body. They act by manipulating caterpillar immune defenses and development, thereby enabling wasps to survive in an immune-competent host. In order to characterize the molecular interactions between *C. congregata* and *M. sexta* the transcriptomes of two immune tissues (fat body and hemocytes) from 24 h parasitized caterpillars were obtained by a high-throughput transcriptomic approach. By this approach we obtained the first functional map of a bracovirus genome and visualized the global impact of parasitism on *M. sexta* immune gene regulation 24 h post oviposition. Results showed that, at this time point, parasitism does not affect the different immune response pathways in the same way. Indeed, parasitism does not prevent activation of antimicrobial peptide signalling pathways, suggesting that the development of wasp eggs and larvae is occurring in an antiseptic environment. In contrast, genes involved in recognition of foreign objects, phenoloxidase activation and cellular immune responses were globally down-regulated after parasitism. This regulation is consistent with the observed inhibition of the caterpillar encapsulation response and allowed to identify potential targets of parasitism implicated in cellular immune responses and melanization.

CONTRIBUTED PAPERS — Tuesday, 10:30-12:30 - ***Bourgueil***

Microsporidia - *Susan Bjornson*

Microsporidia – Emergent Pathogens in the Global Food Chain

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Intensification of food production has the potential to drive increased disease prevalence in food plants and animals. Microsporidia are diversely distributed, opportunistic and density- dependent parasites infecting hosts from almost all known animal taxa. They are frequent in highly managed aquatic and terrestrial hosts, many of which are vulnerable to epizootics, and all of which are critical for stability of the animal-human food chain. Mass rearing and changes in global climate may exacerbate disease and more efficient transmission of parasites in stressed or immune-deficient hosts. Further, human microsporidiosis appears adventitious and primarily associated with an increasing community of immune-deficient individuals. Taken together, strong evidence exists for an increasing prevalence of microsporidiosis in animals and humans and, for sharing of pathogens across hosts and biomes. In this talk, I will review the outcomes of an OECD-funded symposium considering emergence of microsporidiosis in hosts (including humans) from all global biomes. In particular, I will focus on potential for microsporidian infection and disease to act as sentinel for animal and human health relative to exposure to wide ranging environmental stressors (from ocean acidification through to an ageing human population). Further, by focusing on key clades (e.g. *Enterocytozoonidae*), I will consider whether rising burdens of human infection are related to increasing prevalence and intensity of infections in animal populations and, whether opportunism may operate via common nodes of immune suppression in these wide ranging host taxa.

Genome evolution and pre-mRNA splicing in microsporidia and early-diverging fungi

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Microsporidian genomes are among the most reduced eukaryotic genomes. With this reduction has come the loss of many, and in some cases, all of the spliceosomal introns, along with the protein and RNA components of the spliceosome. We previously showed that the few introns of *Encephalitozoon cuniculi* are spliced at low levels, likely as a result of a highly reduced spliceosome that lacks fundamental components, including the apparent loss of the U1 complex. One exception is a relatively long microsporidian intron that is spliced at much higher levels, and has a hyperextended branchpoint motif that we predict increases splicing via enhanced pairing with the U2 snRNA. The motif is conserved in similar introns across all intron-containing microsporidia, although it could not be identified in the sister group to microsporidia, the cryptomycota. Like microsporidia, the cryptomycota are intracellular parasites. Although predominantly known from environmental samples, available cryptomycete genomes do not appear to have undergone the same levels of reduction observed in microsporidia. One member of the group is *Rozella allomycis*, which infects the chytrid, *Allomyces macrogynus*. We are examining the transcriptomes from different life stages of *R. allomycis* to assess splicing levels, and determine if low levels of splicing and extended spliceosomal motifs are ancestral characteristics of microsporidia and cryptomycota. We are also bioinformatically reconstructing the spliceosomes of these lineages. Taken together, these data not only give insight into the evolution of genome reduction and pre-mRNA splicing, but they also increase our understanding of the relationship between microsporidia and other early-diverging fungi.

Molecular phylogenetics as a reason for redefinition of two classical genera of Microsporidia: *Nosema* and *Vairimorpha*

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Nosema and *Vairimorpha* are well-known taxa of Microsporidia infecting representatives of Lepidoptera and other insect orders. The genus *Nosema* is defined as having a monomorphic life cycle with diplosporoblastic sporogony while *Vairimorpha* have both a diplosporoblastic sporogony and a developmental series resulting in uninucleate octospores. Molecular phylogenetic analysis, however, demonstrates that these developmental cycles are not conserved within these taxa, which turn out to be polyphyletic. For example, the original description of *Vairimorpha necatrix* indicates that octospore production is temperature dependent. The additional octosporous sporogony is facultative and cannot serve as a reliable diagnostic character. On the other hand, two distinct clades are revealed among members of these two taxa according to phylogenetic reconstruction using molecular characters. One group is based upon a close relation- ship to *Vairimorpha necatrix* (the type species for the genus *Vairimorpha*) and the other group based upon a close relationship to *Nosema bombycis*, the type species for the genus *Nosema*. The similarity between these two genera does not exceed 85% while similarity among members within clade is not less than 95%. These criteria should be used to draw borders between "true *Nosema* " and "true *Vairimorpha* " and reassign improperly defined taxa, without taking into consideration presence—absence of octosporous sporogony. The research is supported by Grant Council of RF President # MD-4284.2015.4.

Transcriptome and Prokaryotic Expression Analysis of HMG1 of *Nosema bombycis*

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Nosema bombycis, as a typical pathogens of Silkworm Pebrine disease, has the characteristics of eukaryotes, parasites in cell, and always has been as the pathogen to quarantine in silkworm eggs' production. On the basis of the transcriptomics research of *N.bombycis* (GD), Here we selected *N.bombycis* sex related locous genes: *HMG*, *TPT*, *RNA helicase* gene to sequence and do bioinformatics analysis. The main results as follows: 1) 20 nucleotide fragments of *HMG*, *TPT*, *RNA helicase* gene of sex related locus in our database of *N.bombycis* were confirmed. The similarity is more than 90%, respectively. 2) *HMG1* gene (KR057922) was conducted with bioinformatic analysis, the result showed that *N.bombycis* HMG1 protein contains conserved structure domain HMG-box, and also implied that microsporidia exists sexual cycle. Phylogenetic analysis showed that homology of different *N.bombycis* strains was 100%, HMG1 proteins of the same species were highly conserved, and showed that microsporidia and fungi were closely related. 3) In silkworm infected *N.bombycis* developmental periods, *HMG1* has showed different transcription activity. *HMG1* gene has a highest express quantity in the 24hr after p.i. of the forth star silkworm, and in the 72hr without acid treatment eggs , or in 24hr for acid treatment eggs from the p.i. moth, respectively. These results suggested that *HMG1* could be influenced microsporidia reproduction, and involved all procedure of the embryo development of the host. 4) After conducting *HMG1* prokaryotic expression, the MW 21.5 kDa recombinant protein were harvested and prepared for antibody. We successfully detect the HMG1 protein in *N.bombycis*. But we failed to detect fluorescence signal in nucleus. We still less understand HMG1 protein. This work supported by the project of CARS-22-ZJ0205.

Specific nested-PCR and LAMP methods to detect the spore wall protein gene of *Enterocytozoon hepatopenaei* that causes slow growth in penaid shrimp

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As a major world exporter, the Thai shrimp industry has a significant impact on the global market for cultivated shrimp. Just as it is slowly recovering from the catastrophic production drop caused by early mortality syndrome, a newly emerging microsporidian pathogen *Enterocytozoon hepatopenaei* (EHP) is threatening that recovery. Heavy infections of EHP in economically important whiteleg shrimp are associated with slow growth. EHP spread is exacerbated by lack of knowledge of its life cycle, by the environmental resilience of its spores and by lack of any therapeutic agents. The only current means for EHP control is prevention by exclusion from the culture system using rapid, sensitive and highly specific detection methods. However, we found that the currently used diagnostics based on the small ribosomal subunit (SSU) gene can give false positive results due to primer cross reactivity with closely-related microsporidia that do not infect shrimp. This would not only hamper efforts to determine the range of natural reservoir species, but also result in unnecessary rejection of feeds or feed ingredients used for shrimp. To improve diagnostic specificity, a portion of the genome of EHP was sequenced to identify genes that might be targets for more specific molecular diagnostics. As a result, a new nested PCR method and a new LAMP method were developed to target a newly revealed spore wall protein gene of EHP. These methods gave negative results with the microsporidia related to EHP and were thus superior in specificity and sensitivity to the SSU-based methods. Hence, it is recommended that these new diagnostics be used to screen for EHP in feed and environmental samples to detect and prevent entry of EHP into the shrimp cultivation system.

Contributed paper. Tuesday, 11:45, **102**

A pesticide and an intracellular microsporidial parasite target *Drosophila* lipid stores in an unusual "competition" between a natural pathogen and an environmental poison

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We study the host/pathogen interactions between *Drosophila* and a natural intracellular parasite: the microsporidium *Tubulinosema ratisbonensis* (*Tr*). *In vivo*, spores target many tissues, especially the fat body that loses its lipid droplets. Metabolic reserves become depleted during the course of the infection and flies display the hallmarks of severe starvation. Fatty acids supplementation of the infected flies diet increases parasite proliferation and host susceptibility to *Tr*. This effect is blocked when perturbing lipid assimilation and transport originating from the gut. Accordingly, flies fed on nutrient-poor food resist better infection when *de novo* lipid synthesis is blocked, as *Tr* proliferation is hampered. Honeybees exposed to sub-lethal concentrations of the fipronil pesticide succumb earlier to oral microsporidial infection by *Nosema ceranae,* even though its titer is decreased. In flies that have ingested fipronil, we also observed an enhanced susceptibility to *Tr* accompanied by a decrease of its titer. Fipronil also leads to the depletion of the host's lipid reserves. Both the pesticide and the parasite concur to deplete lipid stores, thus accounting for the decreased fitness of infected flies exposed to fipronil and the reduced *Tr* titer. Surprisingly, supplementation of the diet with fatty acids led to an enhanced death rate of exposed flies. While fipronil has been designed to target the nervous system, we have discovered that the ingestion of this pesticide leads to the extrusion of the gut epithelium lipid stores into the lumen, a process that might be responsible for the loss of fat body lipids in a futile attempt of the organism to replenish gut lipid stores.

Contributed paper. Tuesday, 12:00, **103**

***Hyperspora aquatica* n.gn., n.sp. – a hyperparasitic microsporidian infecting paramyxid protists is closely related to crustacean-infecting taxa**

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Centre for Environment, Fisheries and Aquaculture Science (CEFAS), Weymouth, Dorset, United Kingdom

The Paramyxida is an obscure order of parasitic protists within the class Ascetosporea. All characterised ascetosporeans are parasites of invertebrate hosts, including molluscs, crustaceans and polychaetes. Representatives of the genus *Marteilia* are the best studied paramyxids, largely due to their impact on cultured oyster stocks, and their listing in international legislative frameworks. Although several examples of microsporidian hyperparasitism of paramyxids have been reported, phylogenetic data for these taxa are lacking. Recently, a microsporidian parasite was described infecting the paramyxid *Marteilia cochillia,* in cockles (Villalba et al. 2014). In the current study, we investigated the phylogeny of the microsporidian hyperparasite infecting *M. cochillia* in cockles. We show that rather than representing a basally branching taxon in the increasingly replete Cryptomycota/Rozellomycota outgroup (containing taxa such as *Mitosporidium* and *Paramicrosoridium*), this hyperparasite of a paramyxid instead aligns with other known microsporidian parasites infecting aquatic crustaceans. In doing so, we erect a new genus and species (*Hyperspora aquatica* n. gn., n.sp.) to contain the hyperparasite of *M. cochillia* and propose that in this case, paramyxid hyperparasitism may provide a strategy for the vectoring of the microsporidian between hosts of different trophic status (molluscs, crustaceans) in aquatic systems. In particular, it proposes that *H. aquatica* may eventually be detected as a parasite of marine crustaceans. The potential route of transmission of the microsporidian between the paramyxid (in its host cockle) to Crustacea, and, the 'hitch-hiking' strategy employed by *H. aquatica*, will be discussed.

CONTRIBUTED PAPERS — Tuesday, 10:30-12:30 - ***Courteline***

Bacteria 3 - *Baltasar Escriche & Ming Sun*

Contributed paper. Tuesday, 10:30, **104**

Resistance to Bt maize by western corn rootworm: inheritance, fitness costs and cross-resistance

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The western corn rootworm, *Diabrotica virgifera virgifera,* is a serious pest of maize in the United States and is currently managed by planting transgenic maize that produces insecticidal toxins derived from the bacterium *Bacillus thuringiensis* (Bt). Beginning in 2009, field populations of western corn rootworm were identified that had developed resistance to Bt maize producing Cry3Bb1. Subsequent bioassays and field studies found that resistance to Cry3Bb1 also conferred cross-resistance to transgenic corn that produced either mCry3A or eCry3.1Ab. Management of Bt resistance in western corn rootworm has focused on the refuge strategy in conjunction with pyramiding of multiple Bt toxins targeting rootworm. Delays in the evolution of resistance achieved by the refuge strategy are expected to be greater when resistance is inherited as a recessive trait and fitness costs are associated with resistance. Laboratory experiments with strains that contain field-derived resistance alleles indicate that resistance is not inherited in a recessive manner and that few fitness costs may be associated with resistance. It is possible that both of these factors may have contributed to the rapid development of Bt resistance by western corn rootworm. The results of this research are discussed in the context of applying insect resistance management to delay the evolution of resistance by agricultural pests to transgenic Bt crops.

Insect resistance to *Bacillus thuringiensis* Cry3Aa toxin is associated with a novel ABC protein

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The poplar leaf beetle, *Chrysomela tremula*, is a polyvoltine oligophagous beetle responsible for massive attacks on native and introduced hybrid poplars and is one of the major pests of young poplar plantations in France. This beetle is an important model for understanding the mode of action of and resistance mechanism to *Bacillus thuringiensis* toxins in Coleoptera because a Cry3Aa resistant *C. tremula* strain was selected on genetically engineered poplar trees expressing high levels of the Bt Cry3Aa toxin. This strain was derived from an isofemale line established from field-caught insects for which some of the F2 offspring survived on Bt poplar. Resistance to Cry3Aa developed by this species is under the control of a single, completely recessive, autosomal gene. Although the mode of action of Bt toxins is well understood in Lepidoptera, this is not the case in Coleoptera. However, one can assume that, due to high similarities between the 3D structures of lepidopteran-specific and coleopteran-specific Bt Cry toxins, their mode of action may be very similar. We used a larval midgut transcriptome for *C. tremula* as a basis for a candidate gene approach aiming to identify the gene responsible for resistance to Cry3Aa in this species. We will present evidence that a mutation in a novel ABC protein, member of the subfamily B, is genetically linked to Cry3Aa resistance in *C. tremula*.

The limited role of *Bombyx mori* ABCC3 as a Cry toxin receptor in comparison to ABCC2

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The ABC transporter C3 (ABCC3) in lepidopteran insects is a palalog of ABC transporter C2 (ABCC2) that is one of the important receptors of *Bacillus thuringiensis* Cry1A toxin. In recent years, ABCC3 has also been shown to mediate Cry1 toxin intoxication, however, precise toxin specificity and relative contribution of ABCC3 in mediating Cry1 toxin toxicity in comparison to ABCC2 were still unknown. In the present study, we compared toxin specificities, toxin binding kinetics, and expression levels in larval midgut of *Bombyx mori* ABCC2 (BmABCC2) and ABCC3 (BmABCC3). HEK293T cells expressing BmABCC2 showed susceptibilities against Cry1Aa, Cry1Ab and Cry8Ca and did not respond to Cry1Ca, Cry1Da and Cry3Bb. In contrast, HEK293T cells expressing BmABCC3 exhibited susceptibilities against Cry1Aa only and did not respond to Cry1Ab, Cry1Ca, Cry1Da, Cry8Ca and Cry3Bb. The Cry1Aa susceptibility of HEK293T cells expressing BmABCC3 was significantly lower than that of HEK293T cells expressing BmABCC2. Binding analysis revealed that Cry1Aa bound to both BmABCC2 and BmABCC3 whereas Cry1Ab bound to BmABCC2 but not to BmABCC3, indicating that the difference in Cry1Ab toxin specificities between BmABCC2 and BmABCC3 is due to binding characteristics. Binding kinetics of BmABCC2 and BmABCC3 against other Cry toxins were similar to each other but toxin binding did not always correlate with toxin specificities. On the other hand, the mRNA expression levels of *BmABCC2* and *BmABCC3* in midgut of each instar of *B. mori* larvae were similar. These results suggest that BmABCC3 has only a limited role as a Cry toxin receptor because of narrow toxin specificity and lower receptor function against Cry1Aa in comparison to BmABCC2.

Multi-binding ability to functional receptors and BmABCC2 dependent cytotoxicity-relevant property of the domain II loop region of Cry1Aa

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Cry toxins, insecticidal proteins produced by *Bacillus thuringiensis*, have specific toxicity only against narrow groups of insects. This insecticidal specificity is thought to depend on the specific interaction between Cry toxins and insect's receptors, so several candidates of functional receptor have been identified. Above all, cadherin like receptor (BtR) and ABC transporter C2 (ABCC2) seems to be pretty important, because there were some reports about their ability to give insects the resistance to Cry1 toxins when their genes were broken. In this study, we analyzed which regions of Cry1Aa were important for its binding to these receptors and activity through interaction with these receptors. Domain II of Cry1Aa was reported as the binding region of *Bombyx mori* cadherin like receptor (BtR175). In dot blot, binding of Cry1Aa and *B. mori* ABCC2 (BmABCC2) was inhibited competitively by BtR175. This suggests that domain II of Cry1Aa is used in binding with BmABCC2 as well as BtR175. Next, we made some domain II loop mutant toxins and used them in SPR analysis to evaluate binding affinity and in cell swelling assay to evaluate cytotoxicity. The results showed that both the important loop regions for binding to BtR175 and BmABCC2 and for cytotoxicity to cells ectopically expressing these receptors were partially overlapped. These also suggested that binding between Cry toxin and receptors was always necessary but not always sufficient for exerting cytotoxicity. This is the first report showing Multi-receptor binding ability and cell cytotoxicity-relevant property of Cry toxin domain II loop region.

Comparative Analysis of Gene Expression Profiles in Cry1Ac Resistant and Susceptible Strains of *Heliothis virescens*

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The Cry1Ac toxin from the bacterium *Bacillus thuringiensis* (Bt) is the most active Bt toxin against the tobacco budworm (*Heliothis virescens*). In this study, changes in gene expression in the midgut of susceptible (YDK) and Bt-resistant (KCB) strains of *H. virescens* induced by treatment with Cry1Ac were evaluated using RNA-Seq. Fourth instar larvae were fed a sublethal dose of purified Cry1Ac toxin and their midguts were harvested after 0, 120, and 480 min. RNA- Seq libraries were prepared in triplicate using mRNA pools from 3 midguts. Transcripts with differential expression (≥2-fold difference, p≤0.01) were selected for comparing midgut gene expression profiles across different time points within and between strains. Susceptible larvae had 1583, 2073, and 2436 differentially expressed transcripts between 0 and 120 min, 0 and 480min, and 120 and 480 min after

exposure, respectively. In contrast, resistant larvae had 331, 322, and 298 differentially expressed transcripts for the same exposure periods. There were 39, 53, and 9 genes differentially expressed genes in both strains in identical time point comparisons. Differentially expressed genes included reported Cry1Ac receptors such as aminopeptidase N, alkaline phosphatase, and ABC transporter. Overall, ingestion of a sublethal dose of Cry1Ac induced expression of a large number of transcripts in susceptible compared to a Cry1Ac-resistant strain.

Contributed paper. Tuesday, 11:45, **109**

Differential induction of immune system related genes in *Spodoptera exigua* after Vip3Ca challenge

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The beet armyworm *Spodoptera exigua* (H´ubner) is a polyphagous insect pest that causes serious damage to numerous cultivated crops and it is widely distributed around the world. Nowadays, different methodologies are used by growers to control this insect pest such as *Bacillus thuringiensis* products. An earlier study showed that the Vip3Ca protein was little active against *S. exigua* when measuring the mortality parameter but it had a strong effect on the growth inhibition rate. In the present work, the transcript abundance of 51 immune related genes was compared in *S. exigua* larval midguts after Vip3Ca intoxication challenge. The expression profile of the tested genes was not altered when a dose of Vip3Ca that inhibits the growth rate at 95% was used. However, at a dose that inhibits the growth rate at 99%, the expression profile of around 57% of the transcripts tested was modified. In general, the number of genes responding to Vip3Ca intoxication was found lower than in a previous study in which the intoxication was performed with other more active proteins such as Vip3Aa and Cry1Ca. The aminopeptidase N released into the luminal fluid (APN shedding) has been related with midgut damage produced by different agents. This parameter was measured after the exposure of *S. exigua* larvae to a dose of Vip3Aa, Vip3Ca, and Cry1Ca, which inhibits the growth rate at 95%. The results showed that the amount of APN released is higher after Cry1Ca or Vip3Aa exposure than Vip3Ca. Data from the APN shedding assays positively correlated with that observed for the transcripts response. In summary, our results suggest specific transcription response depending on the Bt protein used.

Contributed paper. Tuesday, 12:00, **110**

Microevolutionary mechanisms of wax moth *Galleria mellonella* resistance to *Bacillus thuringiensis*

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Bacillus thuringiensis (Bt) is a widespread Gram positive entomopathogenic bacterium that has been developed as a biopesticide to control pest and vector insects. There is increasing evidence for the occurrence of Bt-resistance among treated insects in the field. In order to explore the mechanisms behind Bt resistance in insects we used the larvae of the Greater wax moth *Galleria mellonella,* which have been established as model hosts for bacterial infections. We experimentally selected a *G. mellonella* line over 20 generations for resistance against Bt. The latter exhibited a 8.8 fold increased resistance when compared with the non-selected line and was used to study immune and stress responses upon challenge with a Bt spore-crystal mixture. The resistant line exhibited differences in innate immune and stress responses as well as in the gut microbiome when compared to the susceptible line. Interestingly, our study elucidated trade-offs, which will be discussed in the light of current insights into mechanisms behind Bt pathogenesis in *G. mellonella*.

Contributed paper. Tuesday, 12:15, **111**

Fitness costs associated with multi-toxin resistance in the cabbage looper (*Trichoplusia ni*)

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Genetically modified (GM) crops producing *Bacillus thuringiensis* (Bt) toxins are used worldwide for agricultural pest control. Current Bt-crops are commonly producing multiple Bt toxins to slow the resistance development to Bt toxins in the field. In previous studies, we have shown that resistance to dual Bt toxin plants (plants with Cry1Ac and Cry2Ab) with two different mechanisms of resistance could be selected in the Lepidopteran *Trichoplusia ni*. Resistance management strategies mostly rely on fitness costs associated with resistance. Although fitness costs associated with Cry1Ac-resistance have been investigated in several different lepidopteran species, the understanding of fitness costs associated with Cry2Ab- and with both Cry1Ac- and Cry2Ab-resistance is limited. We examined the performance of four nearly isogenic strains of *T. ni* with different resistance traits on artificial diet, their preferred host plant (cabbage) and alternative secondary host plants (cotton, tobacco, tomato), to study the impact of the host plant choice on the fitness cost associated with resistance. We also studied the fitness costs in the presence of toxins by rearing *T. ni* on Bt-crops producing one (Cry1Ac) and two (Cry1Ac+Cry2Ab) toxins. This allowed us to analyze the influence of three major parameters of fitness costs of resistance associated with Bt toxins in *T. ni*: the mechanism of resistance, the host plant and the presence of toxins.

WEDNESDAY – 27 July

CROSS DIVISION SYMPOSIUM Wednesday, 08:30-10:30 - *Chinon*

Next Generation Sequencing - *David Bass & Helen Hesketh*

SYMPOSIUM. Wednesday, 08:30 **112**

High-Throughput Sequencing and Bioinformatic Tools for Invertebrate Pathology - an Overview

Ronny Van Aerle
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Over the last decade, advances in high-throughput sequencing technologies have revolutionised environmental research, making it possible for DNA sequencing of non-model organisms of interest to be undertaken. Sequencing approaches are now routinely used in the detection and characterisation of (novel) pathogens, investigation of host-pathogen interactions, and effective development of disease treatment strategies. For the sequencing and identification of pathogens of interest, metagenomics approaches using infected host tissue are frequently used, as it is not always possible to culture these pathogens. High-throughput sequencing can also be used to investigate host-pathogen interactions by investigating (temporal) transcriptomic responses of both the host and pathogen, potentially leading to the discovery of novel drug targets. Environmental samples (e.g. water or soil samples) can be screened for the presence of pathogens using eDNA/meta-barcoding approaches, by which all RNA/DNA conserved regions of rRNA or mitochondrial DNA are amplified and subsequently sequenced. The very significant promise that recent developments in sequencing bring to the field of invertebrate pathology are not devoid of technical challenges, including the need for bioinformatics strategies to analyse the large datasets generated. An overview of the various methods and bioinformatics approaches, including challenges and opportunities for pathogen detection/discovery and studies on host-pathogen interactions will be discussed.

SYMPOSIUM. Wednesday, 09:00 **113**

Next generation sequencing - a powerful approach to assess potential effects of BCAs on microbial communities in soil

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Next-generation sequencing (NGS) has revolutionized research in biology and related disciplines. It has fostered applications in transcriptomics, as well as genomics or metagenomics in previously unexpected dimensions. The massive amounts of data generated by NGS demand efficient bioinformatic pipelines and statistical tools to allow robust conclusions to be drawn. Establishing, developing, and optimizing these approaches has become a research area by itself and tools are rapidly evolving.
Microbial ecology is one discipline in which NGS approaches are applied extensively to investigate microbial diversity and how microbial communities, which fulfill a plethora of important ecosystem services may react to stress factors like climatic change or land management. Applied approaches are based either on random sequencing of metagenomic DNA extracted from a sample or targeted sequencing of marker genes PCR amplified from metagenomic DNA samples. We have applied a targeted sequencing approach to assess potential effects of biocontrol agents (BCA) applications on microbial communities in soil. The experiment will be used as an example to illustrate the targeted NGS approach and the involved bioinformatics and statistical analyses to investigate and explore the data.

SYMPOSIUM. Wednesday, 09:30 **114**

Using dual-RNAseq to study host-pathogen interactions: data generation and analysis

Henrik Hjarvard De Fine Licht
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In any host-pathogen interaction, a central source of information comes from the genes. In contrast to whole genome information that contains sequence information of both genes and non-coding regions, a transcriptome solely provides a genome-wide set of the active genes. In many cases this is an excellent starting point for analyses on a genomic scale. Knowledge about which host and pathogen genes that are active during pathogen infection, can provide new information about the mechanisms of host-pathogen interactions. Active genes are present in the cells as mRNA, which can be sequenced using RNAseq sequencing. My presentation will focus on the practical steps involved with obtaining and analyzing transcriptomes from host-pathogen systems using examples form *Entomophthora muscae* infected houseflies (*Musca domestica*). The dual-RNAseq approach is of particular relevance to host-pathogen systems, because it allows transcriptomes from both host and pathogen to be simultaneously obtained. However, dual- RNAseq also presents additional challenges during analysis and specific advantages and disadvantages of using dual-RNAseq will be discussed.

SYMPOSIUM. Wednesday, 10:00 **115**

Big data and little *Metarhizium*: evolution and interactions of an endophytic insect pathogenic fungus

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In the past two decades, we have witnessed a rapid coevolution of sequencing and computing. The combination of plummeting sequencing costs and the rapid development of bioinformatics for connecting this data back to biology has spurred rapid progress in emerging model organisms, including *Metarhizium* spp. We have embraced the explosive growth of this field to probe the evolution and biology of these fungi, with a focus on understanding their pathogenic (insects) and symbiotic (plant) interactions. We have employed functional gene microarrays to study *Metarhizium* 's microbial interactions at the soil-root interface in natural and managed ecosystems. We are interrogating insect immunity at transcriptomic and genomic (GWAS) levels. We are further employing next-generation genome sequencing technologies to investigate the trophic flexibility and evolution of the versatile life history traits of *Metarhizium* spp. This work has involved comparing very closely related genomes to identify the

mechanisms behind recent shifts in host range and symbiotic potential which has necessarily drawn us into the developing revolution against draft fungal genomes and toward ”perfection” These studies are helping develop entomopathogenic fungi as an important model system for studying/combating infectious processes in general, as well as offering surprising new possibilities for solving insect pest problems.

CONTRIBUTED PAPERS — Wednesday, 08:45-10:30 - ***Bourgueil***

Nematodes - *David Shapiro-Ilan*

Contributed paper. Wednesday, 08:45, **116**

Dissecting the immune defence against the entomopathogenic nematodes: fluctuation of Cecropin and haemocytes during infection of *Spodotera exigua*

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Due to the unique relationship of *S. carpocapsae* with *X. nematophila*, survey on host immune system against monoxenic nematode, symbiotic bacteria and axenic nematode could be an important criteria. Here we measured the expression of cecropin of *Spodoptera exigua* larvae in three status including treated with monoxenic *S. carpocapsae*, axenic *S. carpocapsae* and *X. nematophila* at specified intervals, 2, 4, 8 and 16 hour post injection. The results indicated that monoxenic nematode had the strongest effect on suppression of the insect immune system. The cecropin expression in treated larva with monoxenic *S. carpocapsae* and *X. nematophila* increased to maximum amount at 8 and 4 hour post injection, respectively and then decreased. While, in treated larvae with axenic nematode, the gene expression levels were stable and low. The fluctuation pattern of the cecropin expression were in agreement of cellular response. The results about calculation of haemocyte changes of the larva indicated total haemocytes in treated larva with monoxenic nematode, axenic nematode and symbiotic bacteria at 12, 4 and 8 hour post injection were increased to maximum rate, respectively. Also, the number of granulocytes in treated larva with monoxenic, axenic and symbiotic bacteria at 8, 4 and 2 hour post injection were increased to maximum amount. The results of cellular defense and cecropin expression showed synergistic effect of the nematode and its bacterium in the suppression of insect immune system. The current work provided new insight into expression pattern of AMPs during pathogenesis of bactohelminthic complex. We will discuss about utility of these finding for better understanding mechanisms of the host immunity versus pathogen infection.

Contributed paper. Wednesday, 09:00, **117**

Who one associates with matters:
Role of *Xenorhabdus bovienii* (Enterobacteriaceae) symbionts on the fitness of its *Steinernema* nematode hosts

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The *Steinernema-Xenorhabdus* mutualism is recognized as a powerful model system for studying animal-microbe associations. Studies have shown that *Xenorhabdus* contribute to the fitness of the nematodes. In particular, it has been in relation to nematode virulence and reproduction, which are two keys traits that facilitate the propagation of both symbiotic partners. In this study, we determine the range of symbiont specificity between nine *Xenorhabdus bovienii* strains and three *Steinernema* spp. that are natural hosts of this bacterium. We also evaluated the competitive adaptation of *X. bovienii* to its multiple nematode hosts. Results from these studies showed that the level of specificity between S*. intermedium* and its cognate symbiont (Xbi) is tighter than that observed in the other nematode-bacterium pairs tested. Virulence, reproduction and survival of *S. intermedium* were severely affected when infective juveniles (IJ) associated with non-cognate symbionts. Furthermore, symbiont colonization assays showed that colonization of this nematode by non-cognate bacteria is very low when compared to cognate symbionts. Bacterial competition assays also showed the ability of Xbi to displace other *X. bovienii* strains. Phylogenies developed for *Steinernema*, place *S. intermedium* as a member of a clade that is predecessor to all other species that harbor *X. bovienii* symbionts. Based on this knowledge and evidence provided by this study we speculate that an ancestral *S. intermedium* carrying Xbi may have been able to co-infect an insect host together with another *Steinernema* sp. allowing this bacterium to outcompete other *Xenorhabdu* s sp. and therefore facilitating the subsequent colonization of different *Steinernema* hosts.

Contributed paper. Wednesday, 09:15, **118**

Curative Control of the Peachtree Borer Using Entomopathogenic Nematodes

David Shapiro-Ilan[1], Ted Cottrell[1], Russ Mizell[2], Dan Horton[3]
1 USDA-ARS, United States; 2 University of Florida, United States; 3 University of Georgia, United States

The peachtree borer, *Synanthedon exitiosa* (Say), is a major pest of stone fruit trees in North America. Current management relies primarily upon preventative control using broad spectrum chemical insecticides, especially chlorpyrifos, applied in the late summer or early fall. However, high levels of *S. exitiosa* infestation may still occur through the following spring. Curative treatments applied in the spring would limit damage to the tree. However, such curative measures for control of *S. exitiosa* do not exist. We measured the efficacy of the entomopathogenic nematode, *Steinernema carpocapsae*, as a curative control for existing infestations of *S. exitiosa*. In peach orchards, spring applications of *S. carpocapsae* (obtained from a commercial source) were made to infested trees and compared with chlorpyrifos and a water-only control in 2014 and 2015. Additionally, types of spray equipment were compared: boom sprayer, handgun, or trunk sprayer. To control for effects of application method or nematode source, *in vivo* lab-grown *S. carpocapsae,* applied using a watering can, was also included. Treatment effects were assessed 39 d (2014) or 19 d (2015) later by measuring percentage of trees still infested, and also number of surviving *S. exitiosa* larvae per tree. Results indicated that *S. carpocapsae* provided significant curative control (e.g., > 80% corrected control for the handgun application). In contrast, chlorpyrifos failed to reduce *S. exitiosa* infestations or number of surviving larvae. In most comparisons no effect of nematode application method was detected. In conclusion, our study indicates that *S. carpocapsae* may be used as an effective ”clean-up” measure for *S. exitiosa* infestations.

Contributed paper. Wednesday, 09:30, **119-STU**

Attraction of entomopathogenic nematode isolates to insect feeding is driven by their previous association with wild or domesticated highbush blueberry (*Vaccinium corymbosum*) plants

Monique Rivera, Albrecht Koppenhofer
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Over the past decade, interest in belowground tritrophic interactions involving a plant- root-feeding insect herbivores and entomopathogenic nematodes (EPN) has surged. The novel objective of this study was to investigate differences in this interaction when comparing wild and domesticated plants. We used highbush blueberry (*Vaccinium corymbosum*) as a study system because it was domesticated locally and is produced locally. Two-choice belowground olfactometers were used to examine the preferential attraction of EPN when given three sets of choices: 1. *Anomala orientalis* larvae versus sand only, 2. plants alone and 3. plants with feeding larvae compared to plants alone. Volatiles were collected from the roots of wild and domesticated plants to examine potential signals and variation in volatile profiles between plants with and without feeding larvae. Overall, EPN from domesticated plants (B1) and wild plants (N9) were more attracted to the plant types with which they were originally associated. EPN were not attracted to the larvae alone. Specifically, B1 was not attracted significantly to domesticated or wild plants alone but was attracted to domesticated plants with feeding larvae. In contrast, N9 was more attracted to the wild plants alone but the addition of larvae did not significantly enhance this attraction. Despite some promising leads, volatile analysis did not show significant induction of chemicals from insect feeding on the roots of *V. corymbosum*. Our results suggest that nematodes are locally adapted to the system from which they were isolated but this effect was stronger for domesticated plants and the domesticated nematode isolate.

Contributed paper. **120-STU**
Cancelled

Contributed paper moved to Fungi 2 session. Wednesday, 09:45, **121-STU**

The influence of orchard age on entomopathogenic fungi and nematode population dynamics

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The influence of orchard age on the occurrence and diversity of entomopathogens and their impact on crop pest populations has not been determined. Since the microclimate, tree structure, management systems and soil quality changes as the orchard ages, the ecological processes within the system change. Soil samples were collected from newly planted, juvenile (2 – 4 years) and established (9 years and older) citrus orchards to determine the occurrence and diversity of entomopathogenic fungi (EPF) and entomopathogenic nematodes (EPN). Newly planted orchards had the highest occurrence (38%) of EPF in soil samples, followed by established orchards (34%). EPF occurrence was significantly lower in juvenile orchards (31%) than newly planted orchards. EPN were only found in 2% and 4% of juvenile and established orchard soil samples respectively. False codling moth (FCM), *Thaumatotibia leucotreta*, population numbers were monitored to determine if the percentage occurrence of EPN and EPF found in soil samples correlate with anecdotal reports of higher populations of FCM, during the first three to five harvesting years of citrus planted in virgin soil, after which, FCM numbers seem to decline. Fruit from a juvenile Washington Navel orange orchard were found to be twice as susceptible to FCM as fruit from an established orchard from the same farm. Nutritional analyses showed fruit from the juvenile orchards to attain substantially higher ash content of 3.32% than fruit from established orchards with 0.26% ash content. The influence of these nutritional differences on the susceptibility of FCM larvae to EPF, EPN and Cryptophlebia leucotreta granulovirus (CrleGV) will be determined.

Contributed paper. Wednesday, 10:00, **122**

Potential of entomopathogenic fungi and nematodes against *Parahypopta caestrum* in laboratory assays

Monica Oreste[1], Eustachio Tarasco[1], Luca Ruiu[2]
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Parahypopta caestrum (Hubner) (Lepidoptera: Cossidae) is a highly destructive pest of *Asparagus* spp in Europe. Due to its high destructiveness and the lack of effective control options available, *P. caestrum* can be considered the key pest of *Asparagus* spp. in Italy. The soil-borne larvae bore mines into the roots and the shoots, causing the total destruction of plantations after 2-3 years. The chemical control of this pest is very difficult because chemical pesticides are not able to penetrate into the roots and reach the target. Microbial control agents, as entomopathogenic fungi (EPF) and nematodes (EPN), are able to penetrate the cryptic habitats because they are living organisms and may be horizontally transmitted by infected hosts, so they can be used as alternatives to chemical insecticides against *P. caestrum*. The effect of 6 entomopathogenic nematode strains (Steinernematidae and Heterorhabditidae) and 3 entomopathogenic fungal isolates (*Beauveria bassiana*) was evaluated in laboratory assays against III instar larvae of the Asparagus moth. The results showed that all the nematodes and fungal strains affected the Asparagus moth survival, except the *S. affine* and *H. bacteriophora* strains. *Steinernema feltiae* and *B. bassiana* showed the best performances, killing on average 90% of the *P. caestrum* larvae. Considering the lack of effective chemical control means, the microbial control of the Asparagus moth by EPNs and EPFs reveals promising perspectives and needs further investigations.

Contributed paper. Wednesday, 10:15, **123**

Microbial control of *Cossus cossus* with entomopathogenic nematodes and fungi in laboratory and field assays

Rocco Addante[1], Monica Oreste[1], Angela D'Accolti[1], Luca Ruiu[2], Eustachio Tarasco[1]
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The goat moth *Cossus cossus* (L.) (Lepidoptera, Cossidae) is a wood-boring pest whose larvae bore large galleries under the bark and even deeply into trunks and branches of fruit and forest trees, reducing plant growth and vigour, and causing limbs and branches to fall.Chemical insecticides only have limited efficacy against wood-boring insect pests due to the difficulties in reaching the target. One potential alternative to chemical

insecticides can be the use of microbial control agents, as entomopathogenic nematodes (EPN) and fungi (EPF), because they may be able to penetrate into cryptic habitats and to be horizontally transmitted within the pest populations. Preliminary assays were performed in laboratory conditions in order to evaluate the infectivity of several EPF and EPN autochthonous strains against *C. cossus* larvae of different ages. Results revealed the efficacy of both microbial control agent types in killing the goat moth larvae, although a wide inter- and intraspecific variability in virulence was detected among different microbial strains. Furthermore, a field trial was conducted in infested cherry orchard in Apulia (Italy), using the "live bomb insect" technique (release of pre-infected *Galleria mellonella* larvae in *C. cossus* tunnels), but this approach didn't provide evidence of effective control of *C. cossus* infestation in field conditions.

CONTRIBUTED PAPERS — Wednesday, 08:30-10:30 - ***Courteline***

Virus 4 - *Bryony Bonning & Anne Dalmon*

Contributed paper moved to Special Symposium Tuesday, 09:00, **124-STU**

In-vitro transmission of the Chronic Bee Paralysis Virus and co-exposition with a neonicotinoid in the honeybee

Marianne Coulon[1,2], Frank Schurr[1], Nicolas Cougoule[1], Anne Dalmon[2], Cédric Alaux[2], Yves Le Conte[2], Richard Thiery, Magali Ribiere-Chabert, Eric Dubois
Laboratoire de Sophia Antipolis, Anses, Sophia-Antipolis, France; 2 INRA PACA, Abeilles et environnement, UR406 Avignon, France

Most of honeybee colonies suffer from permanent asymptomatic viral infections that can suddenly prompt substantial mortalities without explanation. However, since neonicotinoids have been shown to reduce bee immunocompetence, we hypothesize that exposure to neonicotinoids could trigger the switch from a covert viral infection to an overt (lethal) infection. We tested this hypothesis by co-exposing bees to the Chronic Bee Paralysis Virus (CBPV) and the neonicotinoid thiamethoxam. First, we developed a method for transmission of CBPV, mimicking the natural route, by exposing healthy bees to previously contaminated bees. This experimental method produced viral loads that were significantly higher than in control bees. This transmission method was then combined with a chronic oral exposition to thiamethoxam, from low to high doses. Emerging bees from a healthy colony were reared in cages following ten conditions: negative control, bees exposed to CBPV, bees exposed to various doses of thiamethoxam, and bees co-exposed to CBPV and thiamethoxam. We followed the mortality and virus level. At lower doses, mortality of the co-exposed bees wasn't significantly different from those exposed to the virus alone. A synergistic effect on mortality was observed for the highest dose of thiamethoxam. However, this synergistic effect wasn't reflected by a significantly higher viral load. Honeybees that were exposed to CBPV held significantly higher viral loads compared to the control bees, but there was no significant difference between control bees and those only exposed to the pesticide. Further studies to investigate potential changes at the immune and detoxification levels would help to better understand such interactions.

Contributed paper. Wednesday, 08:45, **125-STU**

Diversity and evolution of Sinaivirus and related viruses in honeybees and wild hymenoptera

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By providing pollination services, bees are among the most important insects, both ecologically and economically. In this study, we used next generation sequencing technologies to discover and study new insect viruses, potentially harmful for bees. The initial sampling included 30 wild ants (6 species from genus *Messor*) and 13 wild bees (3 species from genus *Halictus*). Individual transcriptomes were sequenced using Illumina technology. A bioinformatic pipeline was developed and allowed transcriptome assembly, protein homology detection with known viruses. Five viral genomes closely related to *Lake Sinaï Virus* (*Sinaivirus*, new genus proposed to ICTV) were discovered in wild ants and bees. After sequence alignments with other known viruses, Maximum Likelihood phylogenies showed that virus found in ants were closely-related to the honeybees infecting LSV-1 and 2. In contrast, viruses found in wild bees were not included in the *Sinaivirus* clade and may correspond to a new viral genus. This species was termed *Halictus scabiosae Associated Virus*. To build a more exhaustive phylogeny of Sinaiviruses, we made a second sampling targeting French and Italian honeybees. Total RNA extraction, RT-PCR of the ORF1/RDRP region and Sanger sequencing were used to detect new *Sinaivirus* sequences. The phylogeny was built from the 42 known *Sinaivirus* sequences, along with 19 new sequences obtained from this study. This analysis revealed the great diversity of the *Sinaivirus* genus, and allowed us to build a robust phylogenetic framework for testing hypothesis of virus transfers between honeybees and wild hymenoptera.

Contributed paper. Wednesday, 09:00, **126-STU**

An opposite effect of *Dicistroviridae* on the RNA interference defense mechanism of their host, *Bombus terrestris*

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Pollinators are plagued by various single-stranded RNA viruses, resulting in complex multi- virus/multi-host networks. Therefore, understanding the way viruses interact with the host's immune system is essential for ensuring pollinator health. The primary antiviral defense system in insects is the RNA interference (RNAi) pathway which is triggered by double-stranded RNA fragments that are generated during viral replication, resulting in the elimination of viral mRNA. Some viruses are known to encode suppressors of RNAi, trying to minimize the effect of this powerful immune pathway. Within the *Dicistroviridae*, a functional suppressor 1A has been found for Cricket paralysis virus (CrPV) in *Drosophila* and predicted in Israeli acute paralysis virus (IAPV). We examined the interaction between these two viruses and the RNAi system of *Bombus terrestris*, which is known to carry IAPV in nature and can artificially be infected with CrPV. We could not confirm the presence of a functional suppressor in IAPV, neither using high definition mass spectrometry, nor through a functionality assay with evaluated the effect of IAPV presence on the silencing efficiency of a reporter gene. Instead of a suppressed RNAi system, an enhancement of the RNAi efficiency was seen, despite only

minimal upregulation of genes involved in RNAi. In contrast, the CrPV 1A proved to be functional in the bumblebee. This disparity between IAPV and CrPV reflects a difference in virulence strategy and may have considerable consequences in the context of multi-virus/multi-host networks.

Contributed paper. Wednesday, 09:15, **127**

Honeybee (*Apis mellifera*) viruses or bee (Apiformes) viruses?

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Viruses are known to contribute to the decline of bee populations. Twenty viruses have been described in *Apis mellifera*, and the most prevalent honey bee viruses have been reported in six other genera of bees. Sharing the same resources may favour spillover of viruses between *A. mellifera* species and wild *bees*. To investigate possible cross-infections, we sampled for two years honeybees and wild bees from blooming *Phacelia tanacaetifolia* with several managed honey bee colonies nearby. More than 1000 specimens from ten genera of wild bees and wasps were collected and checked for the seven most common bee RNA viruses. In *A. mellifera*, *Deformed wing virus* (DWV), *Black queen cell viruses* (BQCV), *Sacbrood virus* (SBV) and *Acute bee paralysis virus* (ABPV) were confirmed to be the most prevalent viruses (> 60% of the specimens), while *Israeli acute paralysis virus* (IAPV), ABPV, and, surprisingly, brood viruses (BQCV, SBV) were the most prevalent in wild species. Phylogenetic studies suggest recent and/or frequent cross-infections, and possible same ancestral origin. Furthermore, 8 wild bee genera were hosting at least three viruses. Thus we infer that "honeybee" viruses may be more generalists than was first evidenced from the managed honeybee colonies.

Contributed paper. Wednesday, 09:30, **128**

Infection dynamics of honeybee viruses in AmE-711 cells

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Honey bees are commonly infected by multiple viruses and understanding of the dynamics of virus infection is important as we strive for improved honey bee health. We used the honey bee cell line AmE-711, along with newly emerged honey bees to examine the dynamics of mixed virus infection. When a mixture of iflavirids [sacbrood bee virus (SBV), deformed wing virus (DWV)] and dicistrovirids [Israeli acute paralysis virus (IAPV), black queen cell virus (BQCV)] was inoculated into either live bees or cell cultures, IAPV replicated to higher levels than other viruses despite the fact that SBV was the major component of the inoculum mixture. When a different virus mix composed mainly of the dicistrovirid Kashmir bee virus (KBV) was tested in cell culture, the outcome was a rapid increase in KBV but not IAPV. Although the AmE-711 cell line was covertly infected with DWV, the presence of this virus did not prevent IAPV and KBV from accumulating to high levels resulting in cytopathic effects. This study demonstrates that multiple mechanisms of virus-host and virus-virus interaction impact virus replication dynamics.

Contributed paper. Wednesday, 09:45, **129**

Occurrence, Pathology, and Ultrastructure of an Iridovirus and Cytoplasmic Polyhedrosis Virus Occurring in Daphnids in the Czech Republic

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Iridoviruses (IVs, family *Iridoviridae*) and cypoviruses (CPVs, family *Reoviridae* ; genus *Cypovirus*) are well known in insects, with thirteen IV species recognized from various orders, and sixteen CPV species known, the latter from species of Lepidoptera. Ironically, an IV and CPV were reported in the daphnid, *Simocehpalus expinosus*, in Florida during 1975, but other reported daphnid virus infections are very rare. Here we report infected daphnids collected from woodland and carp ponds in the Czech Republic, *Daphnia curvirostris* with an IV, and *D. pulex* and *D. ambigua,* with CPVs. IV-infected *D. curvirostris*, about 1% of the population, were more opaque than healthy daphnids and had a slightly bluish tint. Paracrystalline arrays of large, icosahedral virions (243 nm in diameter) occurred in the cytoplasm of enlarged fat cells. The CPV-infections in *D. pulex* and *D. ambigua* were detected by scanning the guts of live daphnids using phase microscopy. Typically infections appeared as spherical clusters of angular occlusion bodies in the cytoplasm of infected cells in less than 1% of the specimens examined. The virions were spherical consisting of a capsid and dense core, characteristic for cypoviruses. CPV virions initially assembled near polyhedra, and had a capsid (67 nm) wider than mature virions (59 nm) occluded in polyhedra. The occurrence of these viruses from distant geographical areas - Florida and the Czech Republic - suggests IV and CPV infections in daphnids may be more widespread than realized, the rarity of reports due to a lack of appropriate surveys.

Contributed paper. Wednesday, 10:00, **130**

In vitro* transcriptomic analyses of the Aphid's secondary symbiont, *Hamiltonella defensa

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In a complex host-parasitoid interaction, the aphid *Acyrthosiphon pisum* is protected from the parasitoid wasp *Aphidius ervi* by a heritable secondary symbiont *Hamiltonella defensa*. Previous work showed that *Hamiltonella*-based protection requires bacteriophages named APSEs that are in family Podoviridae. Multiple strain of APSEs were identified that share a core of conserved genes but are divergent for a variable region consisting of genes coding for holin, lysozyme and toxin genes from three protein families: Shiga-like toxin (Stx) (APSE-1), cytolethal distending toxin (CdtB) (APSE-8), and YD-repeat toxin (APSE-3). These APSEs also differ by their capacity to persist in *H. defensa*, while APSE-8 is a completely integrated provirus in the bacterial genome; APSE-3 exists primarily as an episomal prophage with a low level of lytic replication. Whereas ecological interactions between parasitoid, aphid, bacteria and phage have been studied, molecular interactions have not. Here we developed *in-vitro* method for culturing different APSE and *H. defensa* variants. We also performed a high-throughput transcriptomic analysis

of two *H. defensa* strain carrying APSE (ZA17 with APSE-8 and AS3 with APSE-3) and two *H. defensa* strains without APSE (A2C1 and AS3). Based on these transcriptomes we performed a comparative genomics analysis by identifying SNPs and indels over the *H. defensa* genome for each of the four strains. Our data also identified highly regulated *H. defensa* and APSE genes of functional interest.

Contributed paper. Wednesday, 10:15, **131**

A diverse array of new viral sequences identified in worldwide populations of the Asian citrus psyllid (*Diaphorina citri*) using viral metagenomics

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The Asian citrus psyllid, *Diaphorina citri*, is the natural vector of the bacterium causing Huanglongbing (HLB), or citrus greening disease. HLB and *D. citri* represent a major threat to world citrus production. As there is no cure for HLB, insect vector management is considered one strategy to help control the disease, and *D. citri* viruses might be useful. In this study, we used a metagenomic approach to analyze viral sequences associated with the global population of *D. citri*. By sequencing small RNAs and the transcriptome coupled with bioinformatics analysis, we identified novel viral sequences belonging to the picorna-like virus superfamily, the *Reoviridae*, *Parvoviridae*, and *Bunyaviridae* families, and an unclassified positive-sense single- stranded RNA virus. This is the first comprehensive survey to assess the viral community from worldwide populations of an agricultural insect pest. Our results provide valuable information on new putative viruses, some of which may have the potential to be used as biocontrol agents.

CONTRIBUTED PAPERS — Wednesday, 08:30-10:30 - ***Vouvray***

Microbial Control 2 - *Nina Jenkins*

Contributed paper. Wednesday, 08:30, **132**

The chemical inactivation of H. armigera nucleopolyhedrovirus (HearNPV) on chickpea (*Cicer arietinum*) and other legume crops with studies of its phytochemical mechanism on chickpea

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The use of nucleopolyhedroviruses as commercial biopesticides has grown however these products do have limitations. While products using HearNPV can be effective in controlling *Helicoverpa armigera* on many crops, on some such as the chickpea, its efficacy is limited by a relatively short persistence time. Earlier research has identified that this shorter persistence is due to chemical inactivation of OB on the plant and that isoflavenoids present in the exudate are partly responsible. Laboratory trials on other legumes, the cowpea (*Vigna unguiculata*) and pigeonpea (*Cajunus cajunus*), have shown that while some degree of inactivation occurs on the leaves of these crops it is substantially less than on chickpea. On chickpea while *in vitro* exposure to isoflavenoids were shown to be able to reduce the activity of HearNPV occlusion bodies (OB) this did not account for the full degree of inactivation seen on chickpea leaf surfaces. Tests with other components of chickpea leaf exudate have identified that when the two organic acids present in exudates, malic and oxalic acid are added to the isoflavenoids components the degree of inactivation observed is comparable to that seen on chickpea leaf surfaces. A series of experimental exposures and chemical analyses of chickpea exudates support the hypothesis that the oxalic and malic acids present in chickpea leaf exudates are the more important factors in the surface inactivation of NPV OB on chickpea. The implications of these findings for future research to develop formulations of OB resistant to this chemical inactivation are discussed.

Contributed paper. Wednesday, 08:45, **133**

Temperature effects on time-to-death, *in vivo* production and insecticidal activity of a baculovirus from *Agrotis ipsilon*

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The baculovirus isolated from the black cutworm, *Agrotis ipsilon* (Hufnagel), has potential as a biopesticide to prevent damage to field crops, vegetables and turf grass. Little is known about the impact of temperature on parameters related to production of occlusion bodies (OBs) and subsequent insecticidal activity. Therefore, two isolates, wild-type (WT) and selected egt- (1T6), combined with three temperatures (23°, 28°, and 33° C) for *in vivo* production using 7-d old *A. ipsilon* larvae provided six treatments for comparison. Time-to-death (LT50) was determined for these six treatments during production of the virus. Virus production was measured as OBs per larvae and insecticidal activity (LC50 based on neonate exposure by droplet assay) was subsequently determined for product from each treatment. Warmer temperature during production shortened LT50 values that ranged from 70 h (1T6 at 33° C) to 125 h (WT at 23° C). Larvae exposed to 1T6 died 6.5, 9.3, and 15.0 h faster than larvae exposed to WT at 23°, 28°, and 33° C, respectively. Virus production was not significantly different between the two isolates produced at 28° and 33° C (8.49 × 107 to 2.07 × 108 OBs/larva), but at 23° C WT produced significantly more OBs (2.10 × 108 OBs/larva) than 1T6 (8.57 × 107 OBs/larva). Virus produced at lower temperatures generally had higher insecticidal activity (lower LC50 values) than virus produced at higher temperatures. Thus, virus OBs can be produced faster at higher incubation temperatures (shorter time-to-death), but this benefit may be offset by lower insecticidal activity.

Controlling false codling moth in citrus with a novel Alphabaculovirus, Cryptophlebia peltastica NPV, and a dual isolate Betabaculovirus preparation, Cryptophlebia leucotreta GV

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The false codling moth, *Thaumatotibia leucotreta* Meyrick (Lepidoptera: Tortricidae) is an important pest of citrus and other crops in sub-Saharan Africa. The Cryptophlebia leucotreta granulovirus (CrleGV) has been used successfully for the commercial control of *T. leucotreta* for 12 years. In pursuit of improved microbial control of the pest, a dual isolate CrleGV preparation was produced and tested in field trials in citrus orchards. Selection of the isolates was based on dose-response bioassays conducted in a prior study with six different CrleGV isolates, all originating from South Africa. At 5 x 1013 OBs/ha, efficacy proved superior to that with a commercially available single isolate product, albeit not always significantly. Shortly thereafter, a novel Alphabaculovirus, the Cryptophlebia peltastica nucleopolyhedrovirus (CrpeNPV), was discovered as a homologous infection in the litchi moth, *C. peltastica*, and a heterologous infection in *T. leucotreta*. In a separate study, dose-response bioassays confirmed the virulence of CrpeNPV against *T. leucotreta* and its potential for field control. In field trials at between 5 x 1011 and 5 x 1013 OBs/ha, efficacy of the NPV proved superior to that of the GV. However, this difference was again not necessarily significant. The usefulness of these two novel baculovirus preparations for control of *T. leucotreta* and related Lepidoptera on citrus and other crops is discussed.

Characterization of Helicoverpa armigera nucleopolyhedrovirus in Brazil

Fernando Valicente, Victor Costa, Marcus Soares, Francisco Dimate, Fabrício Morgado, Bergmann Ribeiro
Embrapa Maize and Sorghum, Sete Lagoas, MG, Brazil

Helicoverpa armigera larvae were recently identified in Brazil attacking most of crop areas. More than a 1,300 larvae has been collected in different locations, and observed in laboratory. About 30% of the larvae were parasitized and some dead larvae showed baculovirus symptoms. These dead larvae were macerated individually with distilled water and re-inoculated in 6 days old healthy larvae, reared in laboratory. *H. armigera* larvae are susceptible to Baculovirus, however our major goal was to find different isolates and compare them with the commercially available Gemstar *Q*R. The comparative analysis of the sequencing for the genes LEF-8 and LEF-9 showed that the isolates found in Brazil are closely related to the isolates from Australia and India. All baculovirus isolates tested caused a good mortality rate in larvae of *H. armigera* third instar. However, lethal concentration 50 and lethal time 50 varied among these isolates. All of our isolates showed to be HearNPV and not HzNPV (Gemstar) according to the DNA sequencing. HearNPV -BR2 showed the best results for LC50 e LT50. So, this is the first report of baculovirus isolates infecting *H. armigera* larvae in Brazil, and also the first report of H. armigera baculovirus isolates to be identified as HearNPV. Isolate BR2 is considered for use in *H. armigera* biological control programs due to its characteristcs.

Effect of the Chrysodeixis chalcites single nucleopolyhedrovirus (ChchSNPV) chitinase upon the insecticidal activity of several other alphabaculoviruses

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An isolate of *Chrysodeixis chalcites single nucleopolyhedrovirus* from the Canary Islands has a great potential as a bioinsecticide for the control of *C. chalcites* pest populations on these islands. With the aim of further improving the insecticidal characteristics of this virus the synergetic effect of the homologous chitinase was evaluated in comparison with a commercial enzyme. Chitinases are glycosyl hydrolases present in a wide range of organisms such as bacteria, fungi, plants, arthropods and insect viruses, which degrade chitin. Considerable interest in the chitinolytic enzymes has been generated due to their potential applications as defensive agents against chitin-containing pests and pathogenic organisms, such as insects, nematodes and fungi. Baculovirus chitinases have the ability to degrade the chitin present in the peritrophic matrix of the midgut and the tegument, favouring primary infection and transmission of occlusion bodies from virus-killed insects. In the present study, the chitinase (ChiA) from ChchSNPV was produced in *Escherichia coli* BL21 cells after cloning in the pQE31 expression vector. At the moment different incubation conditions are been testing to optimize ChiA production. We describe the results of bioassays currently in progress to determine the synergistic effect of ChchSNPV ChiA in potentiating the pathogenicity of a number of different alphabaculoviruses.

Alphabaculovirus of *Mamestra brassicae* (Lepidoptera: Noctuidae): Insecticidal activity against several lepidopteran pests

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The high host specificity of baculoviruses has been considered an important factor in the development of these viruses as biological control agents from a biosafety perspective. However, host specificity may represent a disadvantage when a complex of two or more lepidopteran pest species has to be controlled simultaneously, since it requires the use of several virus insecticides. Several studies have reported the host range of many baculoviruses using a qualitative approach (Groner, 1986). However, this information is not enough since quantitative data are necessary in order to determine the insecticidal activity of each baculovirus against the susceptible insect species. The aim of this study was to determine the relative potency of the *Mamestra brassicae multiple nucleopolyhedrovirus* (genus *Alphabaculovirus*) (MbMNPV) against several important lepidopteran pests. The percentage of mortality caused by two different occlusion body (OB) concentrations of MbMNPV (5 x 106 and 5 x 108 OBs/ml) was determined in second instars of six insect species in a preliminary assay. *H. armigera*, *M. brassicae* and *S. exigua* were the most

susceptible species, yielding more than 85% mortality at both concentrations. Intermediate mortalities of approximately 40-60% with the lower concentration and 80-100% with the higher one were obtained in *C. includens* and *S. littoralis*. Finally, *S. frugiperda* showed the least susceptibility to MbMNPV (15% and 55% mortaliaty, respectively). The insecticidal activity of MbMNPV against these pests is being studied in terms of pathogenicity (median lethal concentration (LC50)) and virulence (mean time to death (MTD)). We discuss the potential of MbMNPV as a biological insecticide for the species included in this study.

Contributed paper. Wednesday, 10:00, **138**

Baculovirus synergism: investigating mixed alphabaculovirus and betabaculovirus infections in the false codling moth, *Thaumatotibia leucotreta*, for improved pest control

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Baculovirus based biopesticides have long been shown to be an effective and environmentally friendly approach for the control of agriculturally important insect pests. The false codling moth (FCM), *Thaumatotibia leucotreta* (previously *Cryptophlebia leucotreta*), is a major pest of citrus crops in Southern Africa posing a risk to the export market and thus creating a need for improved control methods. The Cryptophlebia leucotreta granulovirus (CrleGV) has been commercially formulated into the product CryptogranQR and used as part of an integrated pest management program in South Africa for over a decade with much success. A concern is the possibility of resistance developing towards Cryptogran, as was seen in Europe with field populations of *Cydia pomonella* to Cydia pomonella granulovirus (CpGV). In order to prevent such a scenario occurring in South Africa in the case of CrleGV, there is a need to identify and isolate additional baculovirus variants, which can be implemented as new biopesticides. A nucleopolyhedrovirus was recently identified in FCM larval homogenates and genetically characterised. In this study, the NPV was purified from a GV-NPV (alpha-beta-baculovirus) mixture using *C. pomonella* fifth instar larvae. A multiplex PCR assay was developed for the rapid screening of samples. Purified NPV and CrleGV are being evaluated using surface dose bioassays both individually and in various combinations against FCM neonate larvae. Genomic DNA extracted from occlusion bodies purified from larval cadavers will undergo qPCR analysis to determine the GV-NPV ratio resulting from dual infection. A synergistic effect may lead to the development of an improved biopesticide for control of FCM in the field.

Contributed paper moved to MC poster session. Wednesday, 10:15, **139**

Characterization of a Colombian entomopathogenic virus isolated from the sugarcane borer *Diatraea* spp. (Crambidae)

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The *Diatraea* spp. (Lepidoptera: Crambidae) complex is the most important pest attacking sugarcane and some gramineous crops in the Americas. Currently, there are only two records of entomopathogenic viruses isolated from *Diatraea* stem borers, a granulovirus (GV) and a Densovirus (DNV) isolated from *Diatraea saccharalis*. In this sense, the objective of the present work was to identify and characterize an entomopathogenic virus isolated from *Diatraea* spp. larva collected from a sugarcane crop in a previous work. The viral particles were amplified in *D. saccharalis* larvae by droplet feeding method under controlled conditions. Insecticidal activity over second instar *D. saccharalis* larvae was evaluated. Viral morphology was analyzed by transmission electron microscopy and molecular identification and phylogenetic analysis of virus were performed using *lef-8*, *lef-9* and *polh* genes. For the efficacy bioassay, an occlusion bodies (OBs) suspension adjusted to 1x107 OBs/mL showed 63.3% of efficacy, 15 days after treatment. Viral disease symptoms were a significant growth reduction, intense white color and flaccidity without integument liquefaction. The morphology of OBs was irregular ranging between 1.2 and 1.8 μm in diameter with simple and multiple occlusion derived virions (ODVs). The rod-shaped nucleocapsids (nc) were 179-268 nm in length x 21-43 nm in diameter and were in 1-5 nc per virion. Kimura-2-parameter of concatenated *polh, lef-8* and *lef-9* nucleotide sequence grouped this isolate inside *Alphabaculovirus* genera. This is the first report of a multiple nucleopolyhedrovirus isolated from *Diatraea* spp. and the initial characterization of a viral candidate for developing a biopesticide to control the sugarcane borer complex.

BACTERIA DIVISION SYMPOSIUM — Wednesday, 13:30-15:30 - ***Chinon***

Unity and diversity of Entomopathogenic bacteria - *Sophie Gaudriault & David Clarke*

SYMPOSIUM. Wednesday, 13:30 **140**

Insect Pathogenicity Determinants of Plant-Associated Pseudomonads

Christoph Keel†[1], Peter Kupferschmied[1], Maria Péchy-Tarr[1], Céline Terrettaz[1], Pascale Flury[2], María Pilar Vesga Aguado[2], Monika Maurhofer[2]
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Certain plant-colonizing pseudomonads exert varied beneficial activities, which include the suppression of plant diseases caused by pathogenic fungi and protists, the triggering of local and systemic plant defences, and the promotion of plant growth and nutrition. We discovered that phylogenetically defined groups of these plant-beneficial bacteria, notably strains of *Pseudomonas protegens* and *Pseudomonas chlororaphis*, exhibit, in addition, potent oral and systemic insecticidal activities. They are capable of invading with ease the herbivorous larvae of several Lepidopteran and Dipteran plant pest insects. Following oral uptake, they colonize the gut and breach the intestinal epithelial barrier to reach the hemocoel, where they proliferate and ultimately kill the insect. A large insecticidal protein that we termed the Fit toxin functions as a major, but not exclusive, determinant of insect pathogenicity in these pseudomonads. Comparative genomics and targeted mutational analyses allowed us to identify further pathogenicity factors, among them several extracellular products with toxic and lytic activities as well as cell envelope-associated components, notably lipopolysaccharide O-antigens, exopolysaccharides and type VI secretion-related factors. The tailored equipment may allow

these bacteria to colonize certain insects in a highly competitive manner while avoiding the immune defenses of the arthropods, and to switch from a plant- to an insect-adapted lifestyle when opportune.

SYMPOSIUM. Wednesday, 14:00 **141**

Photorhabdus toxins affecting the cytoskeleton

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Photorhabdus species are symbiotically associated with entomopathogenic nematodes. The nematodes invade insect larvae, where they release the bacteria, which then produce toxins to kill the insects. During recent years, we elucidated the molecular mechanisms of some toxins from *Photorhabdus luminescens* and *asymbiotica*. PTC3 and PTC5 are tripartite Tc (toxin complex) toxins from *Photorhabdus luminescens*, which consist of the binding component TcdA1, the linker component TcdB2 and the enzyme components TccC3 and TccC5, respectively. TccC3 ADP- ribosylates actin at Thr148, thereby inducing actin polymerization and aggregation. TccC5 ADP-ribosylates Rho proteins at Gln61/63, resulting in constitutive activation of the GTPases. Rho proteins are also the eukaryotic substrates of *Photorhabdus asymbiotica* toxin PaTox, which causes glycosylation of Rho proteins in tyrosine 32/34. Here, we report on the structure-function relationships of the *Photorhabdus* toxins and discuss the functional consequences of their activities for target cells.

SYMPOSIUM. Wednesday, 14:30 **142**

Photorhabdus Virulence Cassettes: A nano-syringe based toxin secretion and delivery system

Joseph Healey[1], Guowei Yang[2], Isabella Vlisidou[3], Alexia Hapeshi[1], Nick Waterfield[†1]
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The *Photorhabdus* Virulence Cassettes (PVC) represent novel toxin secretion and delivery systems. The PVC needle complex is synthesised by an operon of (usually) 16 genes. Each *Photorhabdus* genome typically encodes around 5 or 6 distinct pvc-operons, each with one or more operon-specific effector protein genes encoded at the 3' end. These typically resemble Type 3 Secretion System (T3SS) effectors. The pvc-operon elaborates a nano-scale contractile 'hypodermic needle' like structure, reminiscent of R-type bacteriocins. However unlike R-type bacteriocins, they are active against eukaryotic rather than prokaryotic cells. PVC proteins show homology to components of both the Type 6 Secretion System (T6SS) and to T4 Bacteriophages. Unlike the T6SS device however, the PVC needle complex is not membrane bound, but is released into the extra-cellular milieu where it subsequently binds to the surface of target cells. We believe that contraction of the syringe structure then leads to the delivery of the pre-loaded effector proteins, into the cytoplasm of the host cell. Since the discovery of PVCs, genomic studies have revealed they are not restricted to the genus *Photorhabdus*. Indeed there seems to be a range of similar devices with varying degrees of homology in a very diverse range of bacteria. Close homologues can be found in examples of *Serratia* (the Anti Feeding Prophage), *Xenorhabdus*, *Yersinia* and *Vibrio*. More diverse homologues can be seen encoded by entirely unrelated genera including *Mesorhizobium*, *Nostoc*, *Pelobacter*, *Shewanella* and the marine worm symbiont *Pseudoalteromonas luteoviolacea*. I will discuss our unpublished work regarding pvc expression and effector protein activity of these amazing molecular devices.

SYMPOSIUM. Wednesday, 15:00 **143**

Comparative genomics in the entomopathogenic genus *Xenorhabdus*: insight into the XaxAB binary cytolysin-encoding locus

Gaëlle Bisch, Jean-Claude Ogier, Sylvie Pages, Anne Lanois, Alain Givaudan, Sophie Gaudriault[†]
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Bacterial infectious process is often deciphered in one model strain inside a taxon. However, the spotlight on a model displays limits. Selecting a strain representative of the whole phylogenetic group may be challenging. Moreover, characterization of diversity in virulence systems may highlights divergent evolutionary strategies of virulence among closed phylogenetic taxa. We will illustrate the interest of decoding virulence systems across taxa, through the example of the XaxAB binary cytolysin-encoding locus initially described in the entomopathogenic strain F1 of *Xenorhabdus nematophila* (*Xn* F1). The *X. nematophila* species is a symbiont of entomopathogenic nematodes living in soils and pathogenic for a broad spectrum of insects by its own. The life cycle of this bacterium involves an alternation between the nematode gut, the hemocoel of the living insect and the insect cadaver. The XaxAB cytolysin is the prototype of a new family of binary toxins encoded by various entomopathogenic bacteria, but also by human and plant pathogens. In *Xn* F1, XaxAB triggers both necrosis and apoptosis in insect cells depending on the cytolysin concentration. Maximal expression of the *xaxAB* locus is observed in larvae cadaver suggesting an important role of XaxAB for *Xn* F1 in this niche. In some other *Xenorhabdus* strains, the *xaxAB* locus is eroded. Interestingly, genomic decay is mediated by different mechanisms (deletion, pseudogenization, ...). Moreover, erosion is frequently associated with *Xenorhabdus* strains attenuated in virulence. We exemplify how these diverse genomic patterns likely reflect different evolutionary scenarios related to different peculiar life cycles inside the *Xenorhabdus* genus.

SYMPOSIUM. Wednesday, 15:30 **144**

Virulence determinants of the beepathogenic species *Paenibacillus larvae*

Elke Genersch
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Like all other beings, honey bees (*Apis mellifera*) are attacked by numerous pathogens. Some of them are just causing covert infections while others are causing overt disease symptoms and even death of individuals and entire colonies. One of the most prominent examples of a deathly honey bee pathogen is the Gram-positive, spore-forming bacterium *Paenibacillus larvae*. This bacterium causes an intestinal infection of honey bee larvae which develops into a systemic infection eventually killing the diseased larvae. This disease is known as American Foulbrood of honey bees (AFB) and is a notifiable disease in most countries. *P. larvae* is an obligate killer because death of larvae and conversion of larval biomass into

bacterial biomass are prerequisites for disease transmission within and between colonies. Hence, *P. larvae* must have evolved effective means to attack larvae, to circumvent the larval immune response and to finally kill and decompose larvae. To understand these processes we aimed at identifying and functionally characterizing *P. larvae* virulence factors. Using comparative genome and proteome analysis, we identified several putative virulence factors. Using gene inactivation strategies in *P. larvae* coupled to different functional assays we experimentally proved the relevance and biological role of these factors and their contribution to *P. larvae* virulence and pathogenesis of AFB. Built upon these findings we developed a model for molecular pathogenesis of *P. larvae* infections furthering our understanding of the pathobiology of this deathly pathogen.

CONTRIBUTED PAPERS — Wednesday, 13:30-15:30 - ***Bourgueil***

Fungi 2 - *Stefan Jaronski & Jørgen Eilenberg*

Contributed Paper, 13:30 **145**

Crude extracts secreted by entomopathogenic mitosporic ascomycetes show potencial for *Ceratitis capitata* (Widemann) (Diptera; Tephritidae) and Drosophila suzukii (Matsumura) (Diptera; Drosophilidae) control

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The insecticidal activity of the crude extract of 12 entomopathogenic fungal strains isolated from different ecosystems was studied against *C. capitata,* a Mediterranean pest, and *D. suzukii,* a new invasive pest. Furthermore, the insecticidal activity of the different fractions (dialyzed, adialyzed, and protein fraction) of the more virulent crude extracts was also studied. The crude extracts from the isolates EAMa 10/07-Su of *Metarhizium guizhouense* and EAPl 10/01- Fil of *Purpureocillium lilacinus* produced high *per os* mortalities against *C. capitata* and *D. suzukii* (100 and 76.7 %, 95.9 and 75 %). The dialyzed fraction of the EAMa 10/07-Su crude extract caused 100 and 59 % mortality of *C. capitata* and *D. suzukii* adults. Nevertheless, the adialyzed fraction of the crude extract of EAPl 10/01-Fil strain was responsible of the insecticidal activity with a 43.3 and 98.3 % mortality of *C. capitata* and *D. suzukii* respectively. Additionally, the protein fraction of the EAPl 10/01-Fil crude extract (PFCE) presented high *per os* activity against *C. capitata* and *D. suzukii* with mortalities values of 83.3 and 70.7 %. Besides, it was demonstrated to be quite thermostable (at 60ºC for 2h and 120ºC for 20 min) and photoresistant (2, 4, y 6 h to 1200 mW/m2 of UV-B radiation). The exposure time to PFCE significantly affected the mortality recorded at 144 h against *C. capitata*, with an increase of the mortality with the exposure time. Finally, the first purification steps of the EAPl 10/01-Fil crude extract showed the protein causing insecticidal activity have an isoelectic point of 4.3. Our results indicate high potential of both the secondary metabolites and the proteins secreted by the EAMa 10/07-Su and EAPl 10/01-Fil strains for the control of *C capitata* and *D. suzukii*

Contributed Paper, 13:45 **146-STU**

Agrobacterium tumefaciens-mediated transgenic *Beauveria bassiana* JEF-007 with reduced virulence against bean bug

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The bean bug, *Riptortus pedestris*, which reduces crop quality and value, is a serious agricultural pest, and chemical pesticides have contributed to the management of the pest, but resistance to these chemicals has significantly limited their use. Herein, we explored how entomopathogenic fungi with different mode of action can potentially be used to control the bean bug, and focused on identifying virulence-related genes. Entomopathogenic *Beauveria bassiana* JEF isolates were assayed against bean bugs under laboratory conditions. One isolate in particular, JEF-007, showed > 80% virulence by both spray and contact-exposure methods. *Agrobacterium tumefaciens*-mediated transformation (AtMT) of *B. bassiana* JEF-007 generated 249 random transformants, two of which (B1-06 and C1-49) showed significantly reduced virulence against *Tenebrio molitor* larvae that were used for rapid screening of virulence-reduced mutants, as well as *R. pedestris* nymphs. The two transformants had remarkably different morphologies, conidial production, and thermotolerance than the wild type. To determine the localization of the randomly inserted T-DNA, thermal asymmetric interlaced (TAIL) PCR was conducted and some genes putatively involved in the virulence of *B. bassiana* JEF-007 in bean bugs were identified. This work provides a strong platform for future functional genetic studies of bean bug-pathogenic *B. bassiana* ; the genes putatively involved in fungal virulence should be experimentally validated by knock-down in future studies.

Contributed Paper, 14:00 **147**

Virulence of commercial strains of *Beauveria bassiana* and *Metarhizium brunneum* against walnut twig beetle adults and impact on brood production

Louela Castrillo[1], John Vandenberg[2], Michael Griggs[2], Robert Camp[3], Bryan Mudder[4], Adam Taylor[3], Albert Mayfield[4]
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Thousand cankers disease (TCD), caused by the walnut twig beetle (WTB), *Pityophthorus juglandis*, and its associated fungal symbiont, *Geosmithia morbida*, is a deadly disease of the eastern black walnut, *Juglans nigra*. Numerous attacks and gallery formation by the WTB and subsequent development of cankers caused by the fungus result in progressive crown dieback, killing affected trees in one to several years following initial infestation. Very few management options are available for preventing or reducing impact of TCD on black walnut trees. Since the development of TCD requires numerous beetle attacks before cankers coalesce and girdle branches, and multiple beetle generations likely result in crown dieback, control strategies that reduce beetle attacks and brood production, without completely eliminating infestation, could still significantly benefit tree health and survival. We are evaluating the use of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium brunneum* against the WTB. Laboratory and field studies conducted in 2014 and 2015 showed that WTB adults are susceptible to commercial strains *B. bassiana* GHA and *M. brunneum* F52. In addition, exposure of the adult beetles to sprayed walnut logs resulted in smaller brood production, with > 80% reduction in subsequent emergence of next generation adults. This ensues from the

death of adults from fungal infection and, subsequently, of their brood from fungal inocula introduced into the gallery or produced by infected parents. These results show the potential use of entomopathogenic fungi in the integrated management of TCD in walnut trees

Contributed Paper, 14:15 **148**

Strategic approach in application of fungal biopesticides: Ecological Biocontrol

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The present issue of insect resistance and environmental toxicity of pesticides is triggering deep discussion about the pest management tactics, in which pest monitoring and control activity are mainly involved. Novel control agents, hopefully overcoming the present issues and problems, should be researched and commercially applied to the farm fields. With the monitoring-based research, additionally we have to focus on the control-based, particularly control agent-based research and application. Entomopathogenic fungi can used as one of the possible novel control agents once considerations are given to the control of soil- or water-dwelling pests. In our research group, the entomopathogenic fungal library has been constructed using the mealworm- based isolation system, which showed a variety of opportunities of their use in pest control. Important key production technologies including granular formulation have been developed to increase their industrialization. Some entomopathogenic fungal isolates showed high biological performance in the control of rice weevils, western flower thrips and Japanese beetles in field stands. To elucidate the fungal mode of action, a fungal transformation system using AtMT and gene identification tools were established. Recently a more deep study about the relationship between insect and entomopathogenic fungi is be investigated using RNA seq. We suggest that to make the entomopathogenic fungal products be applied to agricultural farm field, R&D of down-stream process should be seriously considered as the key step.

Contributed Paper, 14:30 **149-STU**

Natural occurrence of entomopathogenic fungi in apple orchards in Germany related to cropping system and region and evaluation of their efficacy for biocontrol of *Cydia pomonella*

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The BOLNfunded project "Biological control as ecosystem service in integrated and organic pome fruit cultivation" investigates the occurrence and biodiversity of entomopathogenic fungi and evaluates their controlling function as natural antagonists. Therefore, since 2015 soil samples were collected in three of the main apple growing regions in Germany. These regions are located in the north (Altes Land), the centre (Kraichgau) and the south (Lake Constance) of Germany. The samples were collected in organic as well as in integrated managed orchards and additionally in orchards with very few or no pest management or plant protection. Using the practice-proven *Galleria* bait method and a modified version with *Tenebrio molitor*, the soil samples were examined for the occurrence of entomopathogenic fungi. After the isolation, the discovered fungi were tested for their pathogenicity against larvae of the codling moth (*Cydia pomonella*). Subsequently, the effective entomopathogenic fungi were molecularly analyzed for the identification of genus and species. Furthermore, tests were conducted to determine effects of the isolated fungi on invertebrate beneficials. First results indicated that species of the genus *Beauveria* are the most abundant and widespread entomopathogenic fungi in soils of apple orchards in the centre and the north of Germany. However, the genus *Metharizium* could be isolated most frequently in samples of the southern region. In addition few fungi of the genus *Isaria* were found in the first samples of the northern region.

Contributed Paper, 14:45 **150**

Characterization and virulence of *Beauveria bassiana* associated with auger beetle (*Sinoxylon anale*) infesting *Pimenta dioica*

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The incidence of auger beetle, *Sinoxylon anale* Lesne, a destructive pest of cosmopolitan occurrence is reported for the first time on allspice trees, *Pimenta dioica* (L.) Merr. in Kerala, India. The insects bored into fresh twigs from the basal portion resulting in dieback symptoms. The mitochondrial COI gene region of the insect was sequenced. We also isolated an entomopathogenic fungus from infected cadavers of S. anale that was identified as *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales) based on morphological and molecular studies. The fungus was found to grow well in ambient room temperature conditions (28–32 ±2 ◦C) and the infection process on the insect was documented by scanning electron microscopy. Bioassay studies with the isolate indicated that the fungus was highly virulent against adult beetles as evidenced by lower LC50 (2.96 × 106 conidia/ml) and ST50 values (162.2 h at a dose of 1 × 107 conidia/ml and 138.4 h at a dose of 1 × 108 conidia/ml respectively). This is the first record of *B. bassiana* infecting *S. anale* and the fungus hold promise to be developed as a mycoinsecticide against this notorious pest of global quarantine importance.

Contributed Paper, 15:00 **151-STU**

Involvement of Tenecin3 in the infection process of *Tenebrio molitor* by *Beauveria bassiana*

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Beauveria bassiana is a well-known entomopathogenic fungus with a broad host range. Entomopathogenic fungi are used as biocontrol agents, but the time lap they need to control an insect population still limits their use compared to chemical pesticides. A lot of studies aim at increasing the effectiveness of these agents, mostly by focusing on the virulence of the fungi. However, understanding the defenses insect mobilize during the infection process can be key to the improvement of biocontrol strategies. Specifically, the role of the effectors of the insect's immune system during an infection by entomopathogenic fungi remains unclear. The mobilization of these effectors takes place after the recognition of the pathogen and the activation of specific pathways. One of the outcomes of this activation is the production of

antimicrobial peptides (AMPs), whose antibacterial properties have been extensively studied. However, anti- fungal peptide's activity has not attracted a lot of attention so far, especially the *in vivo* activity of such peptides. The mealworm beetle, *Tenebrio molitor*, expresses an antifungal peptide, the tenecin3, which is a glycine-rich peptide, closely related to plant defensins and drosomycin, with no antibacterial activity and of unknown mode of action. Tenecin 3 has been proven to have *in vitro* antifungal activity against *Candida albicans*, but its activity against entomopathogenic fungi has never been studied. We used a gene knock-down approach to study the effect of tenecin 3 on the survival of *T. molitor* after an infection by two different strains of *B. bassiana* and one strain of *B. pseudobassiana*. Interestingly, the mortality pattern varied among the three strains whether tenecin3 was down-regulated or not.

CONTRIBUTED PAPERS — Wednesday, 13:30-15:30 - ***Vouvay***

Virus 5 - *Ikbal Agah Ince & Sassan Asgari*

Contributed paper. Wednesday, 13:30, **152-STU**

Interactions between the salivary gland hypertrophy virus and its host immune system

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Tsetse flies (Diptera; Glossinidae) are naturally infected by *Glossina pallidipes* salivary gland hypertrophy virus (GpSGHV; *Hytrosaviridae*); a large dsDNA virus specifically pathogenic to *Glossina* spp. GpSGHV infections are largely asymptomatic in most of the tsetse species. In *G. pallidipes* asymptomatic infection can convert to symptomatic infection that is characterized by overt salivary gland hyperplasia (SGH). This syndrome also leads to reproductive dysfunction of infected flies. We hypothesised that GpSGHV infection is maintained at low levels by dsRNA-mediated gene silencing, such that only few viral genes are expressed during asymptomatic infections. To test this hypothesis, we first investigated whether host-mediated dsRNA mechanisms are involved in asymptomatic virus infection by comparative analyses of *Argonaute* (*Ago*) and *Dicer* (*Dcr*) gene expression levels in asymptomatic and symptomatic *G. pallidipes*. We found that both *Ago* and *Dcr* were significantly up-regulated in symptomatic compared to asymptomatic flies. Furthermore, short RNA sequence analyses indicated that more small RNAs (19 miRNAs) were produced during symptomatic infections compared to asymptomatic infections (8 miRNAs). When mapped onto the host (*Glossina*) genome, the miRNAs in the asymptomatic flies mapped onto several genes with a putative relation to regulation of transcription, translation, macroautophagy, immunity, apoptosis and tumour suppression. In symptomatic flies, majority of miRNAs mapped to metabolic-related genes and a few to transcription genes. We recently set up knock-down bioassays to investigate the involvement of the miRNA targeted genes in regulating GpSGHV infection in *G. pallidipes* flies.

Contributed paper. Wednesday, 13:45, **153-STU**

Host range of Glossina pallidipes salivary gland hypertrophy virus (GpSGHV)

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The salivary gland hypertrophy virus (SGHV) is a dsDNA virus (family: *Hytrosaviridae*), and It has been reported in many species of tsetse fly (Diptera: Glossinidae). Generally, the virus infection is asymptomatic but in certain tsetse species i.e. *Glossina pallidipes* (Gp), the virus infection can convert to symptomatic and cause the salivary gland hypertrophied (SGH) symptoms. The high prevalence of SGH in tsetse colony is associated with a reduction of flies fecundity and fertility, which may cause colony collapse. To understand the molecular mechanism controlling the development of SGH in Gp and its rare presence/absence in other tsetse species, we attempted to analyse the host range of the GpSGHV in other tsetse species. The GpSGHV virus collected from SGH of Gp and injected into tsetse adults and 3rd instar larvae of Gp, *G. fuscipes* (Gf) *G. brevibalpis* (Gb), *G. p. gambiensis* (Gpg), *G. m. morsitans* (Gmm) and *G. m. centralis* (Gmc) Virus quantification at different times post injection indicated an increase of virus titre in the adults of all injected species except Gb. Dissection of both injected flies and F1 generation showed no development of SGH except *G. pallidipes* F1 generation (46%). Dissection of the flies 10 days post-emergence from injected larvae indicated the presence of SGH in Gp (67 %), Gf (26 %), Gpg (18 %), Gmm (9%), Gmc (6 %) and Gb (0 %). The hypertrophied salivary glands observed in the heterologous species were smaller than SGH normally found in Gp. These results indicate that (i) the GpSGHV can replicate in other tsetse species and (ii) the development of SGH requires a component from immature stages.

Contributed paper. Wednesday, 14:00, **154**

Highjack of intracellular signalling pathways and robust immune responses explain the hytrosavirus-induced differential pathologies in two *Glossina* model species

Ikbal Agah Ince[†1], Henry Kariithi[†2], Sjef Boeren[3], Irene Meki[4,5], Edwin Murungi[6], Everlyne Otieno[6], Steven Ger Nyanjom[6], Monique Van Oers[4], Just M. Vlak[4], Adly Abd-Alla[5]

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Glossina pallidipes salivary gland hypertrophy virus (GpSGHV; *Hytrosaviridae*) is exclusively pathogenic to tsetse flies, vectors of African trypanosomoses. GpSGHV infection is largely latent, but can switch to a symptomatic infection state leading to salivary gland hyperplasia (SGH) and reproductive dysfunction. Of all tsetse species, *G. pallidipes* is the most susceptible to overt SGH symptoms. Whilst in naturally infected *G. pallidipes* SGH occurrence is the exception rather than the rule, SGH is only apparent in the F1 progenies of artificially infected *G. pallidipes*

mothers. Hypothetically, specific host-virus interactions account for the differential GpSGHV pathobiologies observed in different tsetse species and colonies. To test this hypothesis, we used mass spectrometry to investigate GpSGHV-induced protein expression modulations in the salivary gland (SG) proteomes of F1 progenies of two *Glossina* model species, *G. pallidipes* (SGH-susceptible) and *G. morsitans* (SGH-refractory). We identified 540 host proteins, of which 23 and 9 proteins were significantly up and down-regulated, respectively, in *G. pallidipes* compared to *G. morsitans*. We also detected 58 and 5 GpSGHV proteins in *G. pallidipes* and *G. morsitans*, respectively. Whilst *G. pallidipes* had significantly high GpSGHV titres, viral titres in the *G. morsitans* were insignificant, confirming that *G. morsitans* is largely SGH-refractory as compared to *G. pallidipes*. Finally we will discuss how GpSGHV seizes cohorts of intracellular signaling pathways to induce overt SGH in *G. pallidipes*, how robust immune responses block SGH expression in *G. morsitans*, and potential applications of our findings in management of viral infection is insect mass rearing facilities.

Contributed paper. Wednesday, 14:15, **155**

The salivary gland proteome of *Glossina m. morsitans*, parasitized with *Trypanosoma b. brucei*

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Trypanosoma brucei spp, causative agent of African trypanosomiasis, completes metacyclo- genesis (development of mammalian-infective trypomastigotes) in the salivary glands (SGs) of its tsetse vector. Since metacyclic trypomastigotes are largely uncultivable, information on the molecular processes that underpin SG metacyclogenesis is scanty. To bridge this knowledge gap, we employed LC-MS/MS to investigate protein expression modulations in SGs of *T. b. brucei*- infected and uninfected *Glossina m. morsitans*. We identified 361 (host) and 158 (parasite) proteins. Compared to uninfected SGs, the repertoire of the parasitized SG proteome contained proteins that were up-regulated (n = 276), down-regulated (n = 81) or un-modulated (n = 4). Whilst 11.5% (n = 32) of the 276 host proteins were significantly up-regulated, only one of the 81 proteins was significantly down-regulated. Despite high abundance, proteins associated with blood feeding process were down-regulated in parasitized SGs, probably to reduce feeding performance and thus promote vector competence (via increase of biting frequency). Amongst the differentially modulated host proteins in parasitized SGs were also proteins associated with translatome regulation (protein translation, stabilization and degradation), immunity, homeostasis and cytoskeletal traffic. Notable proteins specific to metacyclic trypomastigotes included GPI- anchored surface glycoproteins kinetoplastid calpain, peroxiredoxin AhpC-type, *Trypanosoma* RHS multigene, membrane transporters and molecular chaperone protein families. These data will be discussed in view of strategy development to combat African trypanosomiasis via enhancement of tsetse *Trypanosoma*-refractoriness.

Contributed paper. Wednesday, 14:30, **156**

Characterization of Bustos virus, a new member of the Negevirus group isolated from a *Mansonia* mosquito in the Philippines

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Mosquitoes are known to be important vectors for arthropod-borne viruses (arboviruses), which cause public health issues. In surveillance of mosquito-borne arboviruses, we isolated two distinct viruses from mosquitoes collected in Bustos Bulacan province, Phillipines in 2009, where dengue fever was prevalent. These viruses show rapid replication and strong cytopathic effects in mosquito C6/36 cells. Whole-genome analysis of these viruses demonstrated that both viruses belong to the Negevirus group. One of the viruses, from *Culex vishunui* mosquitoes, is a new strain of Negev virus. The other virus, from a *Mansonia sp*. mosquito, is a new Negevirus designated Bustos virus. Gene expression analysis of the Bustos virus revealed that infected cells contain viral subgenomic RNAs that probably encode proteins from open reading frame (ORF)2 or ORF3. In Bustos virus-infected C6/36 cells, the ORF2 and ORF3 products were distributed in cytoplasm, whereas the ORF1 products formed foci nearby perinuclear region. Purified Bustos virus particles contained at least three proteins, and the major component is encoded by ORF3 and the minor component is encoded by ORF2. Bustos virus did not show infectivity to mammalian BHK-21 cells, suggesting an insect-specific virus.

Contributed paper. Wednesday, 14:45, **157**

RNA activation in mosquito cells and its suppression by the dengue virus NS5 protein

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RNA activation (RNAa) is one of the emerging research areas in molecular biology, which involves small RNAs inducing gene expression by targeting the promoter. Thus far, RNAa has only been found in mammals, including humans, and *Caenorhabditis elegans*, but not in insects. Furthermore, there is no report about the effect of pathogen infection on RNAa. In this study, we employed dsRNA targeting the OpIE2 promoter with the *GFP* gene as the reporter, and checked its effect on GFP expression. In addition to that, the effect of dsRNA to the promoter on GFP expression was evaluated upon dengue virus infection. Our results clearly showed that dsRNA targeting the TATA box of the promoter could induce GFP expression in mosquito cells. In addition, dengue virus, in particular its non-structural protein 5 (NS5) could inhibit the RNA activation. The outcome of this research opens new avenues for RNAa-related research into insect's biology and its potential role in host-pathogen interactions.

Zika virus epidemic in Americas

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Zika virus (ZIKV) is a mosquito-borne RNA virus belonging to the genus *Flavivirus* of the family *Flaviviridae*. ZIKV is transmitted among humans by *Aedes* mosquito species, notably *A. aegypti.* ZIKV can be classified in two genotypes. The African genotype has been found only in the African continent, whereas the Asian genotype has caused outbreaks in Southeast Asia, several Pacific islands and, more recently, in the Americas. In May 2015, Brazil reported autochthonous transmission of ZIKV and since then, Brazil and other countries in Americas have experienced an unprecedented epidemic of ZIKV, reaching an epidemic peak in mid-July 2015 in Brazil. Here, we explore the introduction of ZIKV to the Americas and its subsequent evolution. We based our analyses on several ZIKV genomes, sampled from Brazil and from other South/Central America and Caribbean countries. Phylogenetic and molecular clock analyses show a single introduction of ZIKV into the Americas, estimated to have occurred between May and December 2013, more than 12 months prior to the detection of ZIKV in Brazil. ZIKV genomes from Brazil are phylogenetically interspersed with those from other South/Central America and Caribbean countries suggesting that ZIKV was introduced to Brazil and spread across the Americas afterwards. The results also suggest an early presence of ZIKV in the Northeast states of Brazil where *A. aegypti* is widely present and where Dengue virus is regularly introduced.

New RNA virus producing covert infections in field and laboratory insects of *Ceratitis capitata* (Wiedemann).

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Sequences from three novel RNA viruses (Order: *Picornavirales*) have been discovered from analysis of the transcriptome of *Ceratitis capitata*. One of the viruses constitutes a novel species from a new group in the order, and the other two are members of the *Iflaviridae* family. The abundance of these viruses has been analysed by quantitative PCR in field medflies but also in flies used for the massive production of sterile males for SIT (sterile insect technique) programs. According to our analysis, the majority of the insects from the SIT colonies carry a covert and apparently asymptomatic infection with two of these three viruses, at least in the tested colonies. Moreover, we analysed medflies captured from different areas in the east of Spain and most of the insects were positive for the presence of these viruses. We compared the frequency of infected individual between field captures and massive rearing conditions and we found that in field captures the viral titters were lower, suggesting that the presence of these viruses can be related to a fitness-cost for the insects. The possible effect of these viruses on the biological performance of the males released over the field in the context of SIT programs will be discussed.

CONTRIBUTED PAPERS — Wednesday, 13:30-15:30 - ***Courteline***

Microbial Control 3 - *Sean Moore*

Whole Genome Sequencing of PhopGV Isolates for Control of *Tuta absoluta* in Tomato and *Phthorimaea operculella* and *Tecia solanivora* in Potato

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For the development of new biocontrol agents (BCA) and quality control issues it is important to find genetic markers allowing to discriminate isolates, when working with baculoviruses as active substances in plant protection. Furthermore, a control of pureness and genetically stability of isolates is one approach to answer the call for increasing standards in crop production and thus an reduction of chemical plant protection agents can be reached. Isolates of Phthorimaea operculella granulovirus (PhopGV) were found to infect all of the three pests *T. absoluta*, *P. operculella* and *T. solanivora*. To find a highly virulent isolate to control these three pests, different PhopGV isolates are characterized by biological and molecular means. By analyzing the whole genome data of several PhopGV isolates, produced *in vivo*, the occurrence of PhopGV isolate mixtures was revealed. In contrast, there are single isolates that suppress the replication of others and stayed pure after *in vivo* propagation under presence of a second virus. A line of the host insect *P. operculella* shows a persistent but latent occurrence of one PhopGV isolate over many generations and provides the chance to understand host-virus interactions over time. In addition, a virus-virus interaction of different isolates, that compete and tolerate each other, can be inquired. Hence, PhopGV and *P. opercullella* provides an interesting model to study the plasticity of host response to baculovirus infection. With an increasing understanding of the mechanisms of which baculoviruses and their hosts are underlying, the chances rise to establish new baculovirus based BCA with a consistent efficacy on their target host species. This research was supported by the EU-funded project BIOCOMES.

Identification of a novel mode of resistance against Cydia pomonella granulovirus in codling moth indicates a highly dynamic adaptation in the host population

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The codling moth (CM, *Cydia pomonella*) is the major insect pest in most pome fruit production areas in the world. Commercial products based on Cydia pomonella granulovirus (CpGV, Baculoviridae) are widely used as biocontrol agent of CM. Emergence of resistance of CM populations in Europe to CpGV products have threatened organic apple production as growers rely on effective CM control. First cases of CpGV resistance, with a dominant, sex-linked inheritance, were observed in 2005. This type of resistance is targeted against the viral gene pe38 of CpGVs from phylogenetic group A and is therefore termed the type A resistance. Here, we report on a second type of CpGV resistance, which is targeted against groups A, C, D and E of CpGV (ACDE resistance). Only CpGV isolates genetically grouped to type B can break this newly discovered type of field resistance. By standard genetic crosses and using different CpGV isolates, we showed that the ACDE resistance has a partly different mode of action and is dominant autosomally inherited. It is targeted against some recently registered resistance- breaking CpGV products. From infection experiments and fluorescence-dequenching analyses we concluded that the ACDE resistance is the sum of two independent components, one systemic and one related to midgut factor. By physical mapping we excluded that the ACDE resistance is the consequence of a re-arrangement between the codling moth Z chromosome and an autosome; it is likely caused by another genetic factor, independent from the type A resistance. These new findings urge for the implementation of resistance management strategies when applying CpGV products. This research was supported by Deutsche Forschungsgemeinschaft (grant Je245/14-1).

Entomopathogenic fungi as control agents of *Thaumatotibia leucotreta* in citrus orchards: efficacy and persistence

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Entomopathogenic fungal isolates *Beauveria bassiana* strain G Ar 17 B3 and *Metarhizium anisopliae* strain FCM Ar 23 B3 have been identified as effective control agents of the important citrus pest *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) wandering fifth instars under laboratory conditions. A field trial was conducted to measure the performance of these two isolates, applied via machine spraying prior to peak host larval descent into the soil, in reducing *T. leucotreta* infestation in a citrus orchard under micro-sprinkler irrigation. Further field trials assessing control efficacy and persistence in the soil in an orchard under drip irrigation, was conducted. In comparison to the first field trial, the lower soil moisture measured in the treatment block under drip irrigation was speculated to have negatively influenced the persistence of both isolates and the control efficiency of *B. bassiana* isolate G Ar 17 B3. Regardless, a reduction in *T. leucotreta* infestation was recorded for both isolates following their application at $5x10^{13}$ spores/ha. A further trial assessing the performance of the *B. bassiana* isolate in reducing *T. leucotreta* infestation as a corrective control option (i.e. after a peak in pest fruit infestation and pupation in the soil) was also undertaken. A high percentage reduction in pest infestation of approximately 77% was recorded following the application of this fungus through the micro-sprinkler irrigation system. These results provide evidence and support for the future use of these isolates against *T. leucotreta* thus warranting further investigations.

Control of wireworms in organic potato farming is feasible with an attract-and-kill strategy: technical aspects

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Wireworms are currently the most damaging belowground pest herbivore in potato production systems in Europe. Effective synthetic chemicals used by conventional farmers in the past have been phased out or are no longer marketed; for organic farmers control options are even more limited. In the project ATTRACT, a formulation based on starch and baker's yeast, which releases carbon dioxide over several weeks and which is thus attractive towards wireworms was developed. In the EU project INBIOSOIL this attractive formulation was upgraded with a *Metarhizium brunneum* isolate, which effectively kills the wireworms. This co-formulation acts as a micro-fermenter multiplying the active ingredient, the entomopathogenic fungus, when applied into the soil, thus reducing the dose/hectar and costs. A 10 to 100-fold reduction of the initial biomass concentration in the formulation had no impact on the performance of the formulation. This talk will focus on the technical aspects related to the development and the scale-up of the "Attract-and-Kill"-formulation such as scale-up to technical scale, technology transfer, scale-up to pilot scale and considerations of resources (materials, water, energy), cost efficiency, and application, respectively.

Control of wireworms in organic potato farming is feasible with an attract-and-kill strategy: field trials and mantraps on the way to registration

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As a follow-up to the previous presentation on the development of the "Attract-and-Kill"- formulation this talk will focus on the practical implementation as well as the political and administrative background related to getting the product ATTRACAP registered. Field trials in 2015 demonstrated a mean control efficacy of about 50%. After successful registration, the company BIOCARE (Germany) has just established the production of the formulated capsules with 1000 ha application allowed in 2016. The application costs for the farmers are currently at 300 /ha with a recommended application rate of 30 kg/ha. The problems associated with the implementation and the registration process of ATTRACAP will be discussed in the light of the directive 2009/128/EC of the European Parliament and Council which established a framework for a community action to achieve a sustainable use of pesticides.

Contributed paper. Wednesday, 14:45, **165-STU**

Screen bag formulations (SBF) of entomopathogenic *Beauveria* and *Metarhizium* conidia from granular substrates to control *Riptortus pedestris*

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Most entomopathogenic fungal biopesticides have been produced in the formulations of wet- table powder, liquid concentrate, and emulsifiable concentrate; however, separation of fungal conidia from granular substrates is costly and labor-intensive, which has hampered growth of the fungal biopesticide market. To overcome this challenge, we developed a novel screen bag formulation using mycotized granules from solid cultures of *Beauveria bassiana* JEF-007 and *Metarhizium anisopliae* JEF-003. The two isolates were solid-cultured on Italian millet granules and had a conidial productivity of 1 ~2×10^9 conidia/g. The cultured granules were packed in a screen bag to produce a screen bag formulation and after shaking the formulated bag in water, the number of conidia released into the water was examined and compared to the number released after vortexing the granules in water as a control. To increase the conidial release rate from the bag, various surfactants (Silwet L-77, CO-2.5, CO-12, LE-7, PE-61, TDE-3, Tween 20, and Tween 80) were added to the water followed by assessment of conidial release; Silwet L-77 yielded the highest conidial release. Approximately 50% of conidia were released from the screen bag formulation, and showed similar insecticidal activity against *Riptortus pedestris* nymphs to vortexed conidial suspensions. The screen bag formulation has the advantages of a short production time and low production cost because there is no need for conidial separa tion. Furthermore, the screen bag formulation had insecticidal activity comparable to that of currently used formulations. This formulation technology can be applied to other conidia-based biopesticides, such as fungal fungicides, herbicides, and fertilizers.

Contributed paper. Wednesday, 15:00, **166-STU**

Entomopathogenic fungal library to control *Locusta migratoria* in Korea

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Locust, *Locusta migratoria* (Orthoptera: Acrididae) is one of the outbreaking pests world wide and such big occurrence was recorded in 2014, Korea. However little consideration was given to the management strategy for the pest. Herein we established an indoor locust-rearing system and constructed a locust-pathogenic fungal library to further facilitate the resources to be used as possible biological control agents. A locust colony was provided from the National In stitute of Agricultural Science and Technology and reared in corn or barley plants at artificially manipulated rooms. The critical developmental stages, such as oviposition, hatching and mating were successfully proceeded. Entomopathogenic fungal granules were treated to the locust (2 g/rearing box), and in 5-7 days mycosis was observed in the membranous cuticles of head, abdomen and legs. In particular JEF-003 (*Metarhizium anisopliae*), JEF-186 (*M. lepidiotae*) and JEF-187 (*Clonostachys rogersoniana*) showed high virulence against the locust. A population of locust was exposed to the entomopathogenic fungal conidia-incorporated soil to investigate the possibility of the fungal isolation from natural soil, which resulted in the pathogenesis in 7 ~10 days in laboratory conditions. More than 80% of control efficacy was observed in the greenhouse trial of fungal granular application. This work suggests that locust rearing system was successfully established and entomopathogenic fungi can be used to control the migratory locust.

Contributed paper. Wednesday, 15:15, **167**

Ambrosia beetle mortality and reduced brood production following exposure to microbial control fungi

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The ambrosia beetles Xylosandrus crassiusculus and X. germanus are among the most important exotic pests of orchards and nurseries in the US. Both species have a wide host range and are difficult to control using conventional insecticides because of their cryptic habits. The use of microbial control agents is largely unexplored, but may prove effective by targeting both foundresses and their brood inside tree galleries. Specifically, entomopathogenic fungi could be used to target foundresses, most often the only stage found outside host trees, and mycoparasitic fungi used to target their associated symbiotic fungi. Since most ambrosia beetles feed solely on their associated symbionts, suppression of symbiont growth will deny the developing brood food for survival and limit beetle population increase. The results of our laboratory and field studies showed that both ambrosia beetles are susceptible to entomopathogenic fungi *Beauveria bassiana* and *Metarhizium brunneum*. Further, infected females died prior to egg laying or after producing only a small brood, some of which had up to 100% mortality from fungal infection. Brood infection ensues from fungal inocula introduced into the gallery or produced by infected foundresses. The use of mycoparasitic fungus *Trichoderma harzianum* resulted in beetle galleries with sparse or no symbiont growth, many of which had no or few brood present. These results show the potential of using microbial control fungi in targeting ambrosia beetle populations in the field either directly by killing foundresses or indirectly by suppressing symbiont growth in their galleries to reduce brood production.

Harnessing Metabolites from Entomopathogenic Nematode Symbiotic Bacteria for Broad Use

David Shapiro-Ilan & Selcuk Hazir

SYMPOSIUM. Wednesday, 16:00, **168**

The regulation of secondary metabolism and natural product production in *Photorhabdus*

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Photorhabdus is a genus of bioluminescent, Gram-negative bacterium that is highly virulent to insect larvae whilst, at the same time, maintaining a mutualistic relationship with nematodes of the family Heterorhabditiae. Therefore, *Photorhabdus* is distinguished from other bacteria by its requirement to be both a pathogen and a mutualist of 2 different invertebrate hosts. *Photorhabdus* elaborates an extensive secondary metabolism that is associated with the mutualistic association between the bacteria and the nematode. Amongst the secondary metabolites produced by *Photorhabdus* is a novel antibiotic called 3-5-dihydroxy-4-isopropylstilbene (ST). ST is a stilbene, an important class of bioactive molecule that is normally produced by plants. Indeed, *Photorhabdus* is the only non-plant organism known to produce a stilbene. We have characterized the novel ST-biosynthetic pathway in *Photorhabdus* and we have shown that ST is a multi-potent molecule with roles in both pathogenicity and mutualism. We have also identified a number of factors that are involved in the regulation of ST and secondary metabolism in *Photorhabdus*, including the TCA cycle and the alarmone (p)ppGpp. Therefore, we have begun to characterize the nature of the switch that controls the transition from primary to secondary metabolism (and subsequently pathogenicity and mutualism) in *Photorhabdus*

SYMPOSIUM. Wednesday, 16:30, **169**

Identification and application of eicosanoid biosynthesis inhibitors synthesized by *Xenorhabdus* and *Photorhabdus*

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Eicosanoids are a chemical group of 20 carbon fatty acids that are polyunsaturated and oxygenated. They mediate various insect physiological processes including immune responses. Upon immune challenge, eicosanoids are biosynthesized from phospholipids by a catalytic activity of phospholipase A2 (PLA2) at *sn-2* position to release arachidonic acid (AA). AA is then oxygenated by either cyclooxygenase or lipoxygenase to produce prostaglandin (PG) or leukotrien (LT), respectively. Both PG and LT mediate cellular and humoral immune responses of insects. Entomopathogenic bacteria included in two genera, *Xenorhabdus* and *Photorhabdus*, induce significant immunosuppression of target insects by inhibiting catalytic activity of PLA2 to shut-down eicosanoid biosynthesis. At least 8 different bacterial metabolites that directly inhibit PLA2 have been identified in these two bacterial groups. However, they appear to be synthesized in different times after infection. In addition, they are different in inhibiting immune responses. These suggest that there are different types of PLA2s in insects. These bacterial metabolites significantly enhance pathogenicity of other insect microbial pathogens including *Bacillus thuringiensis* (Bt) by suppressing host immune defense. Mixtures of Bt and the bacterial metabolites are collectively called "Bt-Plus". Different Bt-Plus formulations are currently registered as crop protectants to control lepidopteran, coleopteran, and dipteran insect pests

SYMPOSIUM. Wednesday, 17:00, **170**

Using *Photorhabdus* and *Xenorhabdus* metabolites for control of pecan and peach diseases

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Concentrated metabolites of *Photorhabdus* spp. and *Xenorhabdus* spp. are highly suppressive to important fungal and oomycete pathogens including *Glomerella cingulata*, *Phomopsis* sp., *Phytophthora cactorum*, *Fusicladium effusum*, *Monilinia fructicola*, and *Armillaria tabescens*. Concentrated metabolites or bacterial broth were inhibitory to fungal pathogens *in vitro* and in soil, on leaves and branches of pecan. Biochemical analyses revealed, *trans* -Cinnamic acid (TCA) is a novel bioactive compound produced by *P. luminescens*. Depending on bacterial species, the metabolites showed variation in toxicity to various pathogens. Therefore the efficacy of 10% v/v non-concentrated bacterial filtrates (supernatant) of *X. bovienii, X. nematophila, X. cabanillasii, X. szentirmaii, P. temperata, P. luminescens* (VS) and *P. luminescens* (K22) was evaluated against different fungal plant pathogens. Supernatants of *Xenorhabdus* spp. exhibited stronger suppressive effects on spore germination and vegetative growth compared with *Photorhabdus* spp. Overall, TCA was the most effective treatment at the concentration used. Efficacy of the filtrate varied among *Xenorhabdus* spp. depending on the phytopathogen tested, but *X. szentirmaii* filtrates tended to be most effective. None of the bacterial filtrates, or TCA were phytotoxic. The supernatant was filtered through a 0.22 mm Millipore filter and antifungal compound(s) remained active after autoclaving. The metabolites retained full activity after 7 months storage at room temperature. The results indicate the potential of using TCA or *Xenorhabdus* cell free supernatants as bio-fungicides.

SYMPOSIUM. Wednesday, 17:30, **171**

Natural products from entomopathogenic bacteria - from bugs to the clinic?

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Most antibiotics and several other therapeutics used in the clinic are derived from natural products produced by bacteria and fungi. Despite the importance of such compounds their natural function is often unknown but clearly they are not made originally to cure diseases. Using entomopathogenic bacteria of the genera *Xenorhabdus* and *Photorhabdus* we are trying to answer the following questions: What is the natural function of these natural products? How have these compounds been optimized for what mode of action? How is their biosynthesis regulated?

These typical chemical ecology questions can be addressed since we can study the function of the bacterial natural products in the bacteria alone, together with their nematode host or the insect prey that is infected and killed by the nematodes carrying the bacteria in their gut and we can maintain all levels of this complex life cycle in the lab. Once the basic mechanisms have been identified they can be applied to manipulate the regulatory mechanisms for the production of specific natural products. Moreover, the detailed analysis of several bacterial genomes with their encoded biosynthetic capacity allows the identification of rules for synthetic biology enabling the modification of biosynthesis pathways and even the de novo design of "non-natural" natural products. Furthermore, while addressing these questions we also get access to several of the natural products that can then be tested in different bioassays and the potential of entomopathogenic bacteria as producers of therapeutically useful compounds will be discussed.

CONTRIBUTED PAPERS — Wednesday,16:00-18:00 - ***Courteline***

Microbial Control 4 - *Dietrich Stephan*

Contributed paper. Wednesday, 16:00, **172**

Synergistic combinations of an emulsifiable formulation of *Beauveria bassiana* and a pyrethroid insecticide against insecticide-resistant annual bluegrass weevil, *Listronotus maculicollis*, adults

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The annual bluegrass weevil (ABW), *Listronotus maculicollis*, is a major pest of short- mown golf course turf in the northeastern United States and eastern Canada and has become particularly problematic due to the development of resistance to commonly used insecticides. In the current study, an alternative option to manage highly resistant weevils was explored by combining the pyrethroid insecticide bifenthrin with an emulsifiable formulation of the entomopathogenic fungus *Beauveria bassiana* strain GHA. In the laboratory, the formulated fungus was equally effective against insecticide-susceptible and -resistant adult ABWs at various doses within 3 days after treatment (DAT), and additive or synergistic interactions were observed with fungus-bifenthrin combinations against both populations. In experiments on golf course fairways against insecticide-resistant ABW, strong synergistic interactions were observed. In addition, to determine the insecticidal effects, technical spores of *B. bassiana* GHA and the oil-emulsifiable carrier were tested separately or in combination with bifenthrin. In both separate and combined applications, the oil carrier was responsible for the weevil mortality within 3 DAT, whereas the spores took effect at a delayed time.

Contributed paper. Wednesday, 16:15, **173**

Residual efficacy of *Beauveria bassiana* (Balsamo) Vuillemin, diatomaceous earth, Imidacloprid against three Coleopteran and one psocid species of stored grains

Waqas Wakil[1], Thomas Schmitt[2]
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The residual efficacy of *Beauveria bassiana* sensu lato (Balsamo) Vuillemin, diatomaceous earth alone and in combination with a neonicotinoid insecticide (Imidacloprid) against *Tribolium castaneum* (Coleoptera: Tenebrionidae), *Rhyzopertha dominica* (Coleoptera: Bostrychidae), *Cryptolestes ferrugineus* (Coleoptera: Laemophloeidae) and *Liposcelis paeta* (Psocoptera: Liposcelididae) was studied in laboratory assays. The combine treatments were more effective compared with the alone treatments in controlling the infestation during all storage periods and the highest mortality rates were observed in the grains treated with the combination of DE with Imidacloprid. On the other hand, the mortality of adults for each test insect species was decreased over the storage period of 6 months and the progeny production was increased with the extended storage period. Among the tested insect species *L. paeta* was the most susceptible to all three grain protectants followed by *C. ferrugineus*, *R. dominica* and *T. casteneum*, respectively. The findings of the current study suggest that the use of *B. bassiana*, DE and Imidacloprid as grain protectants may provide control of major stored grain insect species during an extended period of storage.

Contributed paper. Wednesday, 16:30, **174-STU**

Biological Efficacy of the Entomopathogenic Fungi *Isaria fumosorosea* as a Biocontrol Agent Against Pest Insects

Katharina Saar†1, Jasmin Philippi[2], Edgar Schliephake[2], Nicolas Maguire[3], Johannes Jehle[1], Dietrich Stephan[1]
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Entomopathogenic fungi belonging to the species of *Isaria*, *Metarhizium* and *Beauveria* have, due to their lethal effects against several insect pests, a high relevance for the use as a biological control agent (BCA). The EU-funded project BIOCOMES aims on the use of the ubiquitous occurring entomopathogenic fungus *Isaria fumosorosea* in an integrated pest management strategy to control whiteflies (*Bemisia tabaci*) and other pest insects. The selection of highly virulent and effective strains is indispensably in the development of a promising BCA. Mortality effects on whiteflies were investigated in bioassays with different strains of *I. fumosorosea*. Beside this, infected insects showed changes within their feeding and sucking behavior, which were analyzed by using the electrical penetration graph (EPG) method. The principle is based on completion of the electrical circuit when the insect stylet penetrates. Results of the comparisons concerning the changes within the waveform and durations of EPG signals are shown. This observation prefigures that there may also be an influence of *I. fumosorosea* (JKI-BI-1496-01) to the plant virus transmission rate caused by *B. tabaci*. To reach the mentioned aim, several strains of *Isaria* sp., isolated from different geographical origins and hosts, were compared. Firstly, their efficacy and virulence against various pest insect species were tested and investigated by molecular fingerprinting (RFLP/BOX-PCR) to differentiate between the isolates. Thereby, fungi have a large diversity of chitinases, which indicate an interesting starting point to prove impacts of the specificity. A comprehensive study of the chitinases will be done to conclude the relation between virulence and chitinase classes.

Contributed paper. Wednesday, 16:45, **175**

Fungal entomopathogens as endophytes for plant protection: Can they promote plant growth as well?

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There is an increasing evidence that many fungal entomopathogens, almost exclusively researched as insect pathogens, could play additional roles in nature as endophytes, plant disease antagonists, plant growth promoters, and rhizosphere colonizers. *Beauveria bassiana* is the best studied fungal entomopathogen as an endophyte. In contrast, only very few studies have explored the potential of other fungal entomopathogens in the genera of *Beauveria* and *Metarhizium* to occur as endophytes. Also, while most studies investigating the interactions between plants and endophytic fungal entomopathogens have so far focused on the benefits of such interactions to host plants through increased tolerance and resistance to pests and diseases, only a handful of studies to date have investigated the potential role of endophytic fungal entomopathogens as plant growth promoters. Exploring the full potential of interactions between plants and fungal entomopathogens could facilitate a more effective use of these fungi for biocontrol strategies. A series of greenhouse experiments were conducted to examine the potential of several strains of *Beauveria brongniartii* and *Metarhizium brunneum* to endophytically colonize plants by comparing them with a well-known endophytic strain of *B. bassiana*. Our experiments also explore whether the inoculated fungal strains could promote plant growth following their endophytic establishment in plants. Furthermore, our results provide the first report for the plant growth promoting effect in response to increased duration of seed treatment with endophytic fungal entomopathogens.

Contributed paper. Wednesday, 17:00, **176**

Mortality, fecundity and behavior of *Aphis gossypii* Glover feeding on melon leaves endophytically colonized by entomopathogenic fungi

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Various genera of entomopathogenic mitosporic ascomycetes have shown potential for the control of several aphid species by spraying them with the fungal suspensions. However, the recently discovered natural endophytic role of these fungi may allow developing a new systemic biological control method by delivering conidia onto the plant surfaces to achieve an artificial endophytic colonization, which may not only affects aphid survivorship, but also modify life cycle parameters, aphid behavior and stablishment onto the plant, while reducing plant damage. In our study, two isolates of *Beauveria bassiana* and one of *Metarhizium brunneum* with a previously verified endophytic behavior, were assayed against the cotton aphid (*Aphis gossypii* Glover) in surface sprayed and endophytically colonized leaves for lethal and sublethal effects. Dual-choice behavior assays were also developed to elucidate aphid preference to non colonized or colonized melon plants. Aphid mortality rates ranged between 21.6 and 46.0% in leaves superficially contaminated with conidia, and between 23.0 and 44.0 in endophytically colonized leaves. Preliminary results indicate an effect of endophytic colonization neither on aphid fecundity nor on plant selection. These results have to be considered in order to accutely evaluate the efficacy of field sprays with entomopathogenic fungi targeting aphid pests.

CONTRIBUTED PAPERS — Wednesday, 16:00-18:00 - ***Bourgueil***

Disease of Beneficial Invertebrates 2 – *Helen Hesketh*

Contributed paper. Wednesday, 16:00, **177**

Entomopathogens and contaminant microbes of insects for food and feed value chain in Africa

Subramanian Sevgan, Fiaboe Komi, Ekesi Sunday
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Globally ~2,000 species of insects are consumed by about 2 billion people and in Africa where entomophagy is a tradition, it is strongly recommended as a means to enhance food and nutritional security. Edible insects with high levels of proteins also holds potential as alternatives to existing dwindling protein sources for animal feeds. However to harness this potential, consumer confidence needs to be gained by assessing and addressing food safety concerns such as microbiological contaminants of edible insects as fresh, processed and stored products. The potential risk of saprophytic insects to be passive vectors of human and livestock pathogens needs further investigation. Saprophytic fungi belonging to *Aspergillus* spp., *Penicillium* spp, which are mycotoxicogenic and bacteria belonging to *Enterobacteriaceae* are frequently reported on fresh and processed edible insects. For instance, unacceptable levels of mycotoxins have been reported from poorly processed edible insects like mopane worms and stink bugs in Africa. Information on microbial contaminants are critical to establish quality control parameters and inform policy decisions on standards in Africa. Standardization of rearing/improved wild harvesting protocols for edible insects is essential to ensure their continuous supply. Knowledge on entomopathogens that could hinder the production of edible insects and influence their abundance in nature also needs to be assessed. This presentation will take stock of the available information on microbial contaminants and entomopathogens in the insects for food and feed value chain in Africa and discuss recent findings on the same.

Contributed paper. Wednesday, 16:15, **178**

Implications of the honeybee microbial community in the response to major parasites and pathogens

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Honeybee (*Apis mellifera* L.) health is critical to ensure efficient pollination services in natural and agro-ecosystems and for the economical sustainability of hive production systems. Among honeybee parasites and pathogens the mite *Varroa destructor*, a significant virus carrier, and the bacterium *Paenibacillus larvae*, the agent of the American Foulbrood (AFB), represent major concerns in different world areas. Honeybee defense mechanisms include both specific responses at colony level (social immunity) and individual responses like the production of antimicrobial peptides, melanization, phagocytosis and the enzymatic degradation of pathogens. An additional contribution to these innate mechanisms of defense might

be given by the intestinal microbial community, as demonstrated in recent metagenomic works highlighting the antimicrobial properties of different bacterial species associated with this insect. In the present study the down-regulation of immune-related genes (i.e., *Hymenoptaecin*, *Defensin-2*, *Apidaecin*, *PGRP-S1*, *Nimrod-C2*) and the main changes in the microbial community in *Varroa* infested honeybees were studied with a qPCR-based approach, comparing infested and non-infested hives. In addition, culturable bacteria were isolated from field-collected honeybee samples and their antagonism against *P. larvae* was studied in the laboratory. The antimicrobial properties of honeybee isolated *Brevibacillus laterosporus* strains were specifically studied. Funding: this work was supported by Project PRIN 2012, prot. 2012RCEZWH, and by Autonomous regio of Sardinia LR 7/07 TENDER 2013 "API".

Contributed paper. Wednesday, 16:30, **179**

First detection of the Apis mellifera filamentous virus (AmFV) in honey bees (*Apis mellifera*) in China

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There have been increasing reports from beekeepers on the high number of losses of *A. mellifera* colonies in many areas in China. The problem became acute in the spring of 2015 and included the substantial crawling and deaths of honey bees in front of their colonies. The symptoms observed suggested that the honey bees were infected with viral pathogens. Prescreening of samples detected the presence of Apis mellifera filamentous virus (AmFV), a DNA virus that was previously associated with crawling behavior of honey bees in spring. To study its presence in China, we collected samples from 30 apiaries across 10 provinces. We found high prevalences of AmFV infection in honey bee colonies from six provinces. The highest prevalence occurred in Zhejiang (81.25%), and the lowest, in Beijing (30%). Phylogenetic analysis suggested that the Chinese isolate of AmFV was closely related to that reported in the USA. Although the significance of AmFV infection is not fully understood, its presence may be one of the critical factors affecting honey bee health in China. This is the first report of AmFV infection and prevalence in China.

Contributed paper. Wednesday, 16:45, **180**

Trans-generational immune priming in *Tenebrio molitor*: towards the identification of the molecular mechanisms

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While the transfer of immunity from immune-challenged females to their offspring is a well described process in vertebrates, it was thought to be impossible in invertebrates, notably due to the absence of antibody-like systems. Since its first description in bumble-bees in 2001, transgenerational immune priming (TGIP) has been observed in several other invertebrate species. Nevertheless, the molecular mechanisms of TGIP are yet to be elucidated. To elucidate these mechanisms, we focused on the model coleopteran *Tenebrio molitor*. Eggs were collected from females challenged with Gram positive (*Bacillus thuringiensis*) and Gram negative bacteria (*Serratia entomophila*). Global proteomic approach (2D-DIGE, MS/MS) were used to identify proteins differentially abundant between eggs from immune-challenged and from naive *T. molitor* females. The first comprehensive characterization of all antimicrobial peptides (AMPs) present in eggs from immune-challenged females was also conducted. Altogether, these results provide new insights into mechanisms at play in TGIP.

Contributed paper. Wednesday, 17:00, **181**

Molecular cloning and prokaryotic expression of RdRp gene of IAPV

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In this study, a pair of specific primer, according to the complete genomic sequence of IAPV (EF219380), was designed to amplify RNA-dependent RNA polymerase gene (RdRp) from infected honey bee (Apis mellifera) in China. To express RdRp in E.coli, *RdRp* gene was inserted into pEASY-Blunt E1 Expression Vector (TransGen Biotech). And the recombinant plasmid pEASY-RdRp was transfected into BL21 (DE3) cells. The expression product was analyzed with SDS-PAGE and detected the activity of RdRp. The results showed that RdRp was expressed successfully in about 78 kDa and identified the enzyme activity.

CONTRIBUTED PAPERS — Wednesday, 16:00-18:00 - ***Vouvray***

Virus 6 - *Trevor Williams & Martin Erlandson*

Contributed paper. Wednesday, 16:00, **182**

Genotype co-occlusion as a novel paradigm for the development of virus-based insecticides: is the evidence sufficiently convincing yet?

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We have long recognized that insect pathogenic viruses have evolved to be effective pathogens, and not necessarily effective insecticides. Molecular techniques increasingly demonstrate the presence of genetic heterogeneity in populations of entomopathogenic viruses, particularly in baculoviruses. Here, we report on how we have exploited this diversity to develop a novel technology based on four steps: (i) isolate dissection, (ii) genotype interaction analyses, (iii) construction of novel genotype mixtures to create unique non-natural combinations genotypes with improved

insecticidal characteristics (iv) serial passage *in vivo* to refine the transmissibility of genotype mixtures. The production of mixed genotype occlusion bodies (co-occlusion) and mixed genotype occlusion derived virions (co-envelopment) is an integral part of this technology. We show examples of how co-envelopment/co-occlusion can be used for virus insecticide development. This approach has been questioned as it appears to challenge a number of the established models of baculovirus replication and host range. In response, we present the most recent evidence for the physical association between virus nucleocapsids in mixed genotype occlusion bodies, and outline the unique possibilities that this technology represents for baculovirus research and the development of novel biological insecticides.

Contributed paper. Wednesday, 16:15, **183**

Improving infectivity of baculovirus by high-efficiently embedding the enhancing factors into occlusion bodies

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The relative low infectivity of baculoviruses to their host larvae limits their application as insecticidal agent at a larger scale. In this study, a new technique was developed for high- efficiently embedding foreign proteins into *Autographa californica Multiple Nucleopolyhedrovirus* (AcMNPV) occlusion bodies (OBs) and further used to improve its infectivity. A series of recombinant AcMNPV bacmids were constructed by expressing several lengths of the N-terminal of Polyhedrin under the *p10* promoter and the rest C terminal under the *polyhedrin* promoter. The results showed that N-terminal 150 aa and the C-terminal 95 aa of Polyherin under the corresponding promoters could form OBs which embedded ODVs as wild-type AcMNPV. By using this system, when the C-terminal 95 aa Polyhedrin fused with GFP, the recombinant AcMNPV might form OBs with green fluorescent in Sf9 cells. The AcMNPV recombinants were further constructed by fusing the Enhancin or GP37 of granulovirus with the C-terminal 95 aa Polyhedrin and it was found both recombinants formed normal OBs. Bioactivity assays indicated that the median lethal concentrations of these two AcMNPV recombinants were 3 to 5 folds lower than that of the control virus. These results suggested that embedding the enhancing factors in the baculovirus OBs by using this novel technique could significantly improve their infectivity.

Contributed paper. Wednesday, 16:30, **184**

Baculovirus efficacy against the fall armyworm varies with intraspecific genetic variation in soybean defence traits

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Baculoviruses are ingested with the food plants of their insect host. Their ability to establish primary infections in the insect gut can be inhibited by plant defensive chemicals that are activated to combat insect feeding, mainly the oxidation of plant-derived phenolics. This suggests that baculoviruses may be less effective on plants with strong defences. However, since plants among and within species invest differently to a myriad of chemical and physical defences against insects, we hypothesized that some plant genotypes could both strongly resist insects and maintain high baculovirus efficacy. We found that levels of fall armyworm *Spodoptera frugiperda* mortality inflicted by S. frugiperda multiple nucleopolyhedrovirus (SfMNPV) on eight soybean genotypes were reduced to varying degrees when the plant's anti-herbivore defences were induced by the phytohormone, jasmonic acid (JA). JA-induced plant defences did not affect speed of kill or production of virus progeny. Baculovirus efficacy was lower when ingested with foliage that contained higher phenolic content and defensive properties that reduced armyworm weight gain and leaf utilization. In contrast, baculovirus efficacy was high in soybean genotypes that defended the plant by reducing armyworm feeding rate and deterred feeding upon JA-induction. Thus, baculoviruses can be effective on soybean genotypes with strong anti-herbivore defences, provided that the defence against herbivory is through feeding inhibition and deterrence.

Contributed paper. Wednesday, 16:45, **185**

Genetic and biological characterisation of anovel South African Cydia pomonella granulovirus (CpGV-SA) with potential for use in resistance management strategies

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The Cydia pomonella granulovirus (CpGV) has been successfully applied in many countries as a biological control agent in integrated pest management (IPM) programmes for suppression of codling moth populations. However, following prolonged application, resistance to commercial biopesticides based on the Mexican isolate (CpGV-M1) was reported in some *C. pomonella* populations in Europe, leading to a search for novel CpGV variants for managing resistance in the field. A South African CpGV was recently recovered from field collected larvae in an area with no history of baculovirus insecticide application. Full genome sequencing identified CpGV-SA as a genetically distinct isolate with a pairwise identity of 97.2% when aligned against CpGV-M1. In silico restriction profiles of the genome sequence obtained for CpGV-SA and genome sequences of genetically different CpGV isolates originating from Mexico (M1 and M), England (E2), Canada (S) and Iran (I12 and I07) available on the GenBank database confirmed that CpGV-SA is of mixed genotypes. Furthermore, the South African isolate shared the single common difference found in the pe38 gene of resistance overcoming isolates, namely the absence of an internal 24 nucleotide repeat present in CpGV-M1. Biological activity determined by surface dose bioassays was estimated to be 1.632 × 103 OBs/ml (LC50) and 135 hours (LT50) which is comparable with values obtained for CpGV-M (Carpovirusine*Q*R) currently used in South Africa for control of codling moth. This novel CpGV-SA isolate has potential for development as a biopesticide and application in resistance management strategies.

Contributed paper. Wednesday, 17:00, **186**

Baculovirus isolated from *Lymantria dispar* larvae as an example of possible virus adaptation to a new host

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Among the different forest insects, the gypsy moth is a species that is highly susceptible to viral infections. In presented work, baculovirus isolated from gypsy moth dead larvae in Biebrza National Park in Poland (LdMNPV-BNP) shows similar bioactivity to other LdMNPVs used as pest control agents (Gypcheck). However its fully sequenced genome seems to be distant from known LdMNPVs. Genome organization and GC content indicate that LdMNPV-BNP may be recognized as a new species isolated from *Lymantria dispar.* Next-generation sequencing of LdMNPV-BNP revealed gene content (e.g., photolyase) that is not present in any LdMNPV isolate sequenced to date. Because the nucleotide sequences for the *polh*, *lef-8* and *lef-9* genes are very similar to those found in LymoNPV in the GeneBank database, one can draw the conclusion that these two viruses have a common ancestor. Both *Lymantria monacha* and *Lymnatria dispar* moths are present in Biebrza National Park. Caterpillars of these species normally feed on different types of trees (coniferous and deciduous trees, respectively), but during their gradation, when not enough food is present, both can start to consume any available source of food. The isolate characterized in the presented work may be an example of virus transmission and adaptation to a new host on secluded area.

Contributed paper. Wednesday, 17:15, **187**

Genomics of alphabaculovirus isolates infecting *Mamestra* species from North America and Eurasia.

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Alphabaculoviruses infecting *Mamestra configurata* (Bertha armyworm) in North America and *Mamestra brassicae* (cabbage moth) in Eurasia are closely related and complete genomes of several isolates from both host species have been published. The alphabaculovirus isolates from *M. configurata* separate into two species, MacoNPV-A and MacoNPV-B. In contrast those from *M. brassicae*, MbMNPV, are most closely related to MacoNPV-B. The M. configurata and M. brassicae alphabaculovirus isolates appear to have wide host ranges. We sequenced complete genomes of additional isolates that were derived from widely dispersed *M. brassicae* populations from across Eurasia. We show that some of these isolates from *M. brassicae* are MacoNPV-A type viruses. In addition, MacoNPV-B type isolates were also identified but were found to contain some ORFs more closely related to MacoNPV-A homologues. None of the MacoNPV-B type viruses contained a homologue of *lef-7* which is found in all MacoNPV-A type viruses. The most variability in ORF content among the MacoNPV-A genomes is concentrated between *hr1> bro-b* and *bro-b> calyx*. Similarly in MacoNPV-B type genomes, most variation in ORF content is concentrated between *hr1> bro-a* and *bro-b> he65*. Oral infectivity bioassays showed that there were minimal differences in infectivity for MacoNPV-A and MacoNPV-B type isolates in *M. configurata* 2nd instar larvae; while the MacoNPV-B type isolates were typically more infectious for *Trichoplusia ni* 2nd instar larvae than were the MacoNPV-A isolates. The high degree of sequence homology, gene synteny and biological differences between MacoNPV-A and MacoNPV-B type isolates, respectively, suggest a relatively recent evolutionary diversion of these virus species.

Contributed paper. Wednesday, 17:30, **188**

Improved insecticidal activity of Chilo iridescent virus expressing an insect specific neurotoxin

Remziye Nalcacioglu[1], Hacer Muratoglu[1], Aydin Yesilyurt[1], Arzu Ozgen[1], Zihni Demirbag[†1], Van Oers Monique[2], Vlak Just[2]
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Previously we have generated a recombinant Chilo iridescent virus (CIV), by placing the green fluorescent protein gene (gfp) to the CIV 157L open reading frame (ORF) locus and have shown that the obtained recombinant was fully infectious both in cell culture and in larvae. This study opened us new venues toward valuable strategies for increasing the viral pathogenicity of CIV by inserting virulence genes into its genome. In this study, to improve the viral pathogenicity, we constructed a recombinant CIV (rCIV-D157L/gfp-AaIT), by replacing the 157L ORF with the AaIT neurotoxin gene from the scorpion *Androctonus australis* and the gfp gene under individual viral major capsid protein (mcp) promoters. Recombinant virus was purified by successive rounds of plaque purification in cell culture. The infectivity of rCIV-D157L/gfp-AaIT was compared to the wild-type and rCIV-Δ157L-gfp, CIV, in *Spodoptera frugiperda* (Sf-9) cells. One-step growth curves for recombinant and wild-type CIVs were similar. Toxin expression in infected third instar *Galleria mellonella* larvae was confirmed by western blot analysis using an antibody against the AaIT protein. Recombinant virus caused a rigid paralysis in the infected *G. mellonella* larvae two days after injection. Bioassays on these larvae demonstrated that the speed of action (LT50) and pathogenicity (LC50) of the recombinant virus were strikingly enhanced compared to wild-type CIV. These results suggest that this recombinant form of CIV provides further opportunities to develop a commercial product to control susceptible pest insects.This study was supported by The Scientific and Technological Research Council of Turkey (TUBITAK, Project No. 113Z748).

Contributed paper. Wednesday, 17:45, **189**

Characterization of the Baculovirus-Densovirus interaction when co-infecting the same host

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Accumulating evidences suggest that mixed viral infections are abundant in the nature, yet studies trying to understand virus interactions when co-infecting the same host are rare. Such studies might be indispensable to have clear view about the possible interactions between pests and viral enemies when designing new biopesticides. Among insect viruses, Baculoviruses (BVs) are widely

studied and used in biological control. In addition, the interest on other viruses such as Densovirus is increasing. The Junonia coenia densovirus (JcDNV) is highly pathogenic to a wide range of lepidopteran species sharing the same hosts with different BVs. Therefore, we sought to study the interaction between JcDNV and different BVs co-infecting the same host. In a first experiment, we co-infected the cell line Hi5 with JcDNV and Autographa californica multiple nucleopolyhedrovirus (AcMNPV). While the AcMNPV replication was not affected, JcDNV replication was significantly reduced. In an *in vivo* model, experiment was performed co-infecting *Spodoptera exigua* larvae with a dose of its native BV (SeMNPV) killing about 70% of the total larvae and a sub-lethal dose of JcDNV. When larvae are simultaneously infected with both viruses, larval mortality reached 95%. However, when larvae were consecutively infected with SeMNPV and then with JcDNV, similar mortality to that caused by SeMNPV alone was obtained. Nevertheless, when the larvae are previously infected with JcDNV, the mortality of larvae declined. Since, the simultaneous infections seem to increase the effectiveness of the infection, we are aiming to obtain occlusion bodies that co-occlude BV and JcDNV viral particles as a possible strategy for more efficient viral control of the lepidopteran pests.

THURSDAY – 28 July

VIRUS DIVISION SYMPOSIUM

Thursday, 08:00-10:00 - ***Descartes***

Viruses and horizontal gene transfers - *Elisabeth Herniou & Jean-Michel Drezen*

SYMPOSIUM. Thursday, 08:00, **190**

Mechanisms of horizontal gene transfer in metazoans.

Chiara Boschetti, Isobel Eyres, Alastair Crisp, Elton Gargioni Grisoste Barbosa, Timothy Barraclough, Gos Micklem, Alan Tunnacliffe
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Horizontal gene transfer (HGT) is the transfer of genetic material from one organism to another that is not a direct descendant. HGT is widespread among bacteria and plays a significant role in their evolution, but was thought to be extremely rare among metazoans, which instead were believed to inherit all their genes directly from their parents (vertical gene transfer). This is not the case in bdelloid rotifers, however, where an astonishing 10% of active genes have originated through HGT. Recent work has further shown that, contrary to the accepted paradigm, HGT is not limited to bacteria and few small invertebrates, but is widespread in metazoans, occurring in all species analysed, although to different degrees. We might now ask how genes are transferred among organisms characterised by a segregated germline and with a different genetic environment from the donor organism, which is often microbial, and what effects HGT has on animal evolution. I will explore these topics and the current hypotheses underlying the mechanisms which, according to the latest data, organisms use to increase their genetic variability and adaptability to changing environments.

SYMPOSIUM. Thursday, 08:20, **191**

Evidence of recent interspecies horizontal gene transfer regarding nucleopolyhedrovirus infection of *Spodoptera frugiperda*

Mariano Belaich[1], Gloria Barrera[2], Manuel Patarroyo[3], Laura Villamizar[2], Pablo Ghiringhelli[1]
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Baculoviruses are insect viruses carrying large dsDNA genomes with significant applications such as pest control, recombinant protein production, gene delivery in mammals or as a model of DNA evolution. These pathogens have high species diversity, which is expressed in their diverse properties including morphology, host range, virulence or pathogenicity. Besides, this diversity is associated with a differential gene content that include 37 encoded core sequences within a set of 90-180 open reading frames (ORFs). Therefore their genomes could be divided into two main parts, the essential DNA (encoding crucial factors) and the auxiliary DNA (encoding collaborative factors), plus the trans-genome represented by the host which contributes with other important activities. All these considerations reveal the plasticity of baculoviruses and the importance of horizontal gene transfer (HGT), including recombination and transposition events. *Spodoptera frugiperda* (Lepidoptera: Noctuidae), represents a significant pest for agriculture; it is a host for baculoviruses such as the nucleopolyhedrovirus of *Spodoptera frugiperda* (SfMNPV) (Colombia strain, genotype A) having been classified as a Group II alphabaculovirus making it a very attractive target for bioinsecticidal use. The analysis of its genome revealed that 2 ORFs were not included in the other reported genotypes for this species (SfMNPV-NicB, SfMNPV- NicG, SfMNPV-19 and SfMNPV-3AP2). An in-depth bioinformatics study showed that these sequences were acquired by a recent homologous recombination process between Spodoptera frugiperda and Spodoptera litura nucleopolyhedroviruses, revealing the importance of HGT in the evolution of baculoviruses.

SYMPOSIUM. Thursday, 08:40, **192**

Continuous Influx of Genetic Material from Host to Virus Populations

Gilbert Clément[1], Jean Peccoud[1], Aurélien Chateigner[2], Bouziane Moumen[1], Richard Cordaux[1], Elisabeth Herniou[2]
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2 Institut de recherche sur la biologie de l'insecte, CNRS UMR7261, Université François Rabelais, Tours, France

While gene exchange is known to occur between viruses and their hosts, this phenomenon has never been studied at the level of the viral population. Here we report that each time a virus from the Baculoviridae family infects a moth, a large number (dozens to hundreds) and high diversity of moth DNA sequences (86 different sequences) can integrate into replicating viral genomes. The majority of the moth DNA sequences are transposable elements (TEs, n=69) belonging to 10 superfamilies of DNA transposons and three superfamilies of retrotransposons. In addition to *bona fide* DNA transposition, we uncover microhomology-mediated recombination as a mechanism explaining integration of moth sequences into viral genomes. Many sequences integrated multiple times at multiple positions along the viral genome. We detected a total of 27,504 insertions of

moth sequences in the 21 viral populations and we calculate that on average, 4.8% of viruses harbor at least one moth sequence in these populations. Finally, we found that at least 21 of the moth TEs integrated into viral genomes underwent repeated horizontal transfers between various insect species, including some lepidopterans susceptible to baculoviruses. Our results identify host DNA influx as a potent source of genetic diversity in viral populations. They also support a role for baculoviruses as vectors of DNA HT between insects.

SYMPOSIUM. Thursday, 09:00, **193**

Microplitis demolitor bracovirus DNAs integrate into the genome of host cells

Michael Strand
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Bracoviruses (Polydnaviridae) are large, double-stranded DNA viruses that have evolved into mutualists of insects called parasitoid wasps. As part of this mutualism, wasps inject bracoviruses into insects they parasitize, which results in expression of viral gene products that wasp offspring require for survival. Several lines of evidence indicate that the circular DNAs in bracovirus virions integrate into the genome of host cells they infect. Microplitis demolitor bracovirus (MdBV) genomic DNAs harbor imperfect direct repeats that identify where DNAs integrate while analysis of MdBV virions identify several features that could play roles in regulating integration. Here I discuss progress on understanding the mechanisms underlying how and where MdBV DNAs integrate in the genomes of host cells. The potential significance of integration for BV function in parasitism will also be discussed.

SYMPOSIUM. Thursday, 09:20, **194**

Acquisition and Domestication of bracoviral genes in *Spodoptera* spp contributes to their defense against pathogens

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Horizontal gene transfer (HGT) in eukaryotes is now recognized as an important factor in evolution for the acquisition of novel functions from other species. Bracoviruses are symbiotic viruses associated with tens of thousands of species of parasitic wasps that develop within the body of lepidopteran hosts. Once in the host body, bracoviruses infect lepidopteran hemocytes inducing the expression of their specific immunosuppressive proteins that are essential for successful development of the wasp larvae within lepidopteran hosts. We show here that in several lineages, lepidopteran genomes have repeatedly acquired genes from bracovirus, leading to the production of lepidopteran lineages that harbour bracovirus sequences. In the case of *Spodoptera spp*, two groups of genes derived from bracovirus were found integrated in their genomes, the *BV2-5* (*Gasmin*) and several *C-type lectins*. Functional analysis of the Gasmin protein revealed its ability to interfere with actin polymerization, taking part in cellular processes related with the host response to viral and bacterial infections. Similarly, functional analysis of the C-type lectins also showed that some of them could contribute to shape the host susceptibility to natural pathogens. Those results support that acquisition of bracovirus sequences by Lepidoptera has resulted in the domestication of several genes that result in adaptive advantages for the host. We hypothesize that bracovirus-mediated HGT has played an important role in the evolutionary arms race between Lepidoptera and their pathogens.

CONTRIBUTED PAPERS — Thursday, 08:00-10:00 - ***Courteline***

Fungi 3 - *Nicolai Vitt Meyling & Annette Bruun Jensen*

Contributed paper. Thursday, 08:00, **195**

Molecular characterization of icipe EPF isolates: opportunities and challenges

Fathiya Khamis[†], Nguya Maniania, Komivi Akutse, Levi Ombura, Subramanian Sevgan, Sunday Ekesi
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Icipe hosts a microbial germplasm at its Headquarters in Nairobi, Kenya, with entomopathogenic fungi (EPF) isolates accounting for 80% of the collection. Among the various EPF isolates, 4 *Metarhizium anisopliae* isolates have been commercialized and several others are at various stages of development. Currently, biossays are being undertaken to screen different isolates of EPF for virulence against a variety of horticultural and pulse pests such as *Tuta absoluta*, *Aphis gosypii*, *Brevicoryne brassicae*, *Lipaphis peudobrassicae*, *Maruca vitrata,* etc; for incorporation into IPM strategies for their management. However, there are no specific markers that have been developed for monitoring and tracking these isolates applied as biological control agents. The current study aimed at developing specific probes, molecular or otherwise for monitoring and tracking the fate, establishment and potential environmental risks of the different commercially- available isolates of *M. anisopliae* and those with commercial prospects. The information generated should also be valuable for the evaluation of the overall efficacy and safeguarding against unauthorized use by potential commercial competitors. The study characterized the isolates in terms of their virulence through the expression of the chitinase gene both *in vitro* and *in vivo*, and the challenges and opportunities through the use of other tools for tracking the isolates.

The comparative analysis of defense reactions and midgut microbiota of *Galleria mellonella* under development of mycoses caused by *Metarhizium robertsii* and *Ordyceps militaris*

Olga Yaroslavtseva†[1], Oksana Tomilova[1], Alex Pervushin[2], Natalia Kryukova[1], Ecatherine Chertkova[1], Olga Polenogova[1], Maxim Tyurin[1], Ivan Dubovskiy[1], Viktor Glupov[1], Vadim Kryukov[1]

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Entomopathogenic fungi use various strategies in interactions with hosts. We studied pathogenesis caused by entomopathogenic fungi with a different pathogenic strategy: *Metarhizium robertsii* (Mr) and *Ordyceps militaris* (Cm). Injection of hyphal bodies in *Galleria mellonella* larvae was used to compare both insects immune response and development of fungus in haemocoel. Speed of kill was slow after Cm injection compare with Mr. However, strong suppression of cellular immunity was registered under the injection with Cm against to Mr. Infection with Mr induced apoptosis while Cm led to necrosis of hemocytes. The dramatic increase of dopamine level in hemolymph was registered during pathogenesis caused by Cm compared to Mr. The activity of phenoloxidases inhibited more significantly under development of Mr compared to Cm, but in activity of detoxification enzymes contrary tendency was observed. Infection with Cm and Mr resulted in different changes in midgut bacterial community of the wax moth. Infected with Mr led to increasing of diversity indexes due to increase of subdominant taxons portion (*Advenella, Roseburea, Brevundimona, Porphyromonas*) and decreasing of dominant species share (*Enterococcus*). Contrariwise, infected with Cm led to decrease of diversity indexes due to mass development of dominating species (*Enterococcus*). The significant differences in the insect defense reactions and midgut microbiota during Mr and Cm infections confirm the various strategies used the fungi to manipulate by host immunity during colonization. The study was supported by the Russian Federation President grant -6278.2015.4.

The histone deacetylase HosA regulates cell cycle, conidiation, virulence and stress tolerance in *Beauveria bassiana*

Qing Cai, Sheng-Hua Ying, Ming-Guang Feng†

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Histone deacetylases can control gene expression by modulating the accessibility of chromatin to transcription factors. Here, we characterized HosA, one of the histone deacetylases in *Beauveria bassiana*. Deletion of *hosA* resulted in disturbed cell cycle, which was represented by a remarked delay in G1/S transition. In D*hosA*, hyphal cells were elongated due to affected septation pattern, accompanied with depressed transcript levels of many septation-dependent genes. The D*hosA* mutant suffered a suppression of ˜50% in either aerial conidiation or submerged blastospore production, and most of its conidia and blastospores were significantly enlarged. The deletion mutant was also more sensitive to oxidative and osmotic stresses during normal cultivation but exhibited an increase in conidial tolerance to UV-B irradiation. Virulence was attenuated in D*hosA* versus wild-type against *Galleria mellonella* larvae, which were infected by either topical application or intrahemoceol injection of a standardized conidial suspension. All changes were restored to wild-type levels by targeted *hosA* complementation. These results indicate a significant role for HosA in regulating cell cycle, conidiation and pathogenicity and hence in contributing to biological control potential of *B. bassiana* against insect pests.

A novel vacuolar protein is required for the *in vitro* asexual cycle and full virulence of *Beauveria bassiana*

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Many hypothetical proteins (HPs) exist in genomic databases of entomopathogenic fungi, and their unknown functions block deep insights into molecular mechanisms involved in fungal development, host infection and environmental adaptation. Here we characterized an HP, which was found localizing in the vacuoles of *Beauveria bassiana* and hence was named as vacuolar protein (VacP). Blast analysis revealed an existence of VacP orthologues in many insect and plant pathogenic fungi. Deletion of *vacP* in *B. bassiana* resulted in a reduction of 80% in aerial conidiation capacity and of 96% in submerged blastospore production, accompanied with a significant delay in conidial germination and conspicuous defects in cell cycle. In standardized bioassay, the deletion mutant lost approximately half of virulence to *Galleria mellonella* larvae infected by topical application or intrahaemocoel injection of conidial suspension. All changes were restored by targeted *vacP* complementation. Taken together, VacP is required for *in vitro* asexual cycle and full virulence in *B. bassiana*.

Characterization of high osmolarity glycerol pathway essential for environmental adaptation in *Beauveria bassiana*

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High osmolarity glycerol (HOG) pathway required for multiple stress responses in budding yeast comprises mitogen-activated protein (MAP) kinases. In this study, we established a markerless transformation system of *Beauveria bassiana* by means of uridine auxotrophy and used the system for deleting the genes encoding the MAP kinase Hog1, the MAP kinase kinase Pbs2, and the MAP kinase kinase kinases Ssk2 and Ste11. Colony growth in 1/4 SDAY was completely inhibited for D*ssk2*, D*pbs2* and D*hog1* in response to NaCl (1 M) or sorbitol (1.5 M), which caused the same response of D*ste11* as wild-type. Aside from the hypersensitivity to high osmolarity, most deletion mutants became much more sensitive to oxidation, heat and UV-B irradiation but were more resistant to fludioxonil fungicide and cell wall perturbation by Congo red. Exceptionally, D*ste11* and wild-type were equally responsive to fludioxonil and Congo red. All the mutants exhibited severe defects in aerial conidiation, germination and virulence except for unaffected conidiation in D*pbs2*. The phosphorylation signal of Hog1 as a hallmark of the HOG pathway was detected only in

D*ste11* and wild-type but became undetectable in D*ssk2*, D*pbs2* and D*hog1* under osmotic, thermal and oxidative stresses. Taken together, the Ssk2-Pbs2-Hog1 cascade acted as the HOG pathway in *B. bassiana*. However, the Ste11 was seemingly not involved in the fungal HOG pathway.

CONTRIBUTED PAPERS — Thursday, 08:15-10:00 – ***Bourgueil***

Diseases of Beneficial Invertebrate 3 - *Grant Stentiford*

Contributed paper. Thursday, 08:15, **200**

Applications of environmental DNA (eDNA) methods in parasitology

David Bass[1,2], Georgia Ward[3], Rose Kerr[1], Catherine Troman[2], Corey Holt[1], Kelly Bateman[1], Beth Okamura[2], Grant Stentiford[1]
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Environmental DNA (eDNA) is a conceptual term referring to a wide range of methods associated with sequencing DNA or RNA from 'environmental' samples. We define 'environmental' samples as ranging from conventional samples such as sediment, soil, and filtered water, through animal/plant associated microbiomes (gut flora, rhizosphere, faeces, etc.), to organismal tissue. In terms of the methods and analyses involved there is no logical break between these extremes. The nucleic acids sequenced may be amplicons generated by general or specific PCR, or shotgun approaches: (meta)genomes and (meta)transcriptomes. These methodologies have mostly so far been used to explore or compare biodiversity of free-living organisms, but increasingly parasites and pathogens are the focus of eDNA studies, and parasites detected during the course of more general studies are being analysed more thoroughly. This talk will discuss the utility of eDNA methods in parasitology and pathology, illustrated by a wide range of case studies from specifically-targeted experiments to larger-scale ecology- and discovery-based work.

Contributed paper. Thursday, 08:30, **201**

Exploring into an emerging disease in the circum-Antarctic keystone predator, the sea star *Odontaster validus*

Laura Núñez Pons[1,2,3], Thierry Work[4], Robert Rameyer[4], Juan Moles[3], Carlos Angulo-Preckler[3], Conxita Avila[3]
1 Smithsonian Tropical Research Institute (STRI), Panama; 2 Hawai Institute of Marine Biology (HIMB), University of Hawaii at Manoa, United States; 3 Universitat de Barcelona, Spain; 4 US Geological Survey, National Wildlife Health Center, Honolulu, United States

Recent devastating syndromes that have annihilated sea stars populations along the American Pacific coasts have raised awareness of unusual mortality events in echinoderms globally. Echinoderms are key components in benthic biocenoses for top-down and bottom-up regulations, thus any affection on their populations may have important knock-down consequences on whole ecosystems. During the Antarctic cruises ACTIQUIM-3 (Dec 2011-Feb 2012) and -4 (Jan-Mar 2013) at Deception Island, we documented clinical signs of multifocal ulceration affecting ~10% of the population of the keystone predator *Odontaster validus* Koehler, 1906. Histological analyses were performed on healthy and affected specimens demonstrating ulceration of epidermis associated with inflammation and tissue necrosis in diseased animals. To characterize homeostatic disorders in the microbiome of Bacteria/Archaea and Fungi (ITS-1) associated with disease, we compared the microbial communities in healthy and diseased specimens, using MISeq technology to barcode 16S V3–V4 and ITS-1 regions. The analysis of several divisions within the microbiota provides information about interactions and compositions related to healthy and diseased stages. This will assist to disentangle the roles different microbial compositions play in marine ecosystems, in particular in the much less understood Southern Antarctic Ocean. This is a preliminary study of a deeper exploration into this emerging disease affecting the Antarctic benthos. Future efforts will focus on coordinating additional analyses at the microscopic and ultrastructural level with microbial and molecular studies, to gain greater insights as to potential etiologies of this disease.

Contributed paper. **202-STU**
Cancelled

Contributed paper. Thursday, 08:45, **203-STU**

Impact of water temperature on immune-related gene expression in American lobster experimentally infected with White Spot Syndrome Virus

Louise-Marie Roux[†1,2], Philip Byrne[2,3], Fraser Clark[1], Spencer Greenwood[1]
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American lobster (*Homarus americanus*) inhabit ocean waters that fluctuate in temperature between 0 °C and 20 °C. Variable water temperatures pose biological challenges for lobster, as processes such as development, growth and reproduction are temperature dependent. The full impact of these temperature variations on the immune response of American lobster is unknown. White spot syndrome virus (WSSV) is one of the most virulent pathogens threatening shrimp aquaculture and can infect a wide range of other crustacean hosts. In an earlier study, adult American lobster produced a targeted immune response to WSSV, following intramuscular injection of the virus at 20°C. More recent work suggests that American lobster can only become infected with WSSV following an unnatural route of exposure (intramuscular injection) in permissively warm water conditions. This study used a similar WSSV injection challenge model to explore the molecular immune response of American lobster held at 4 temperatures (10 °C, 15 °C, 17.5 °C, 20 °C). A lobster specific microarray was used to monitor transcriptomic changes across 14,592 genes during viral infection at the different temperatures. A total of 383 genes were significantly differentiated across the temperature groups, based on hepatopancreas mRNA. Genes of interest include crustacean immune-related genes such as crustin, thioredoxin, SAA, and PGE2. RT-qPCR was used for gene expression confirmation. Overall results from this study are helping to characterize temperature dependent changes in lobster immunity.

Development of a duplex PCR as screening tool for the detection of Crangon crangon bacilliform virus in the European brown shrimp Crangon crangon.

Benigna Van Eynde[†1,2], Olivier Christiaens[1], Daan Delbare[2], Kelly Bateman[3], Grant Stentiford[3], Annette Dullemans[4], Monique Van Oers[5], Guy Smagghe[1]

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The brown shrimp *Crangon crangon* is an important species ecologically and economically. Due to low landings of large animals (less than 2 % > 7 cm) and a growing demand for living shrimps, *C. crangon* was identified as a candidate for aquaculture. However, in 2004, researchers found that this species could be infected with C. Crangon Bacilliform Virus, an intranuclear bacilliform virus mainly infecting the hepatopancreas. This virus, acting as an extra stress factor, could affect the health status of *C. crangon* reared in captivity, resulting in higher mortality. CCBV could therefore be the reason why the life cycle of C. crangon was never closed in captivity. A screening tool for the detection of CCBV was developed in order to examine the infection severity. The research focused on the optimization of a duplex PCR, which amplifies an internal amplification control gene and a viral gene. Once the duplex PCR was optimized with reared shrimp, a prevalence study was conducted with wild samples from several regions of the Belgian Coast. Results indicate that the prevalence of CCBV is between 80 and 94 % in the examined populations. The high prevalence can indicate that the virus is transmitted from female to offspring. To confirm this hypothesis, more experiments are needed to determine how this virus is transmitted to the offspring. The development of a detection tool for CCBV offers the ability to rapidly and reliably screen wild and reared *C. crangon* and also transmission can be studied in more detail. This will enable us to select healthy and virus-free individuals, which will not only contribute to the rearing of this species, but also for research purposes, allowing us to use standardized bioassay tests.

Microbial patters allied to coral disease and bleaching, insights from Kane'Ohe Bay – Oahu (Hawaii)

Laura Núñez Pons[1,2,3], Raphael Ritson-Williams[2], Emilia Sogin[2], Ross Cunning[2], Anthony Amend[2]

1 Smithsonian Tropical Research Institute (STRI), Panama; 2 Hawai Institute of Marine Biology (HIMB), University of Hawaii at Manoa, United States; 3 Universitat de Barcelona, Spain

Marine reefs are ecologically complex biogenic formations, and the fitness of the building blocks, the scleractinian corals, is key for the health of the whole ecosystem. Disease in animals involves an interaction between agent, host and environment. In reef corals however, one of the major threats globally is the effect of climate warming, causing bleaching through the loss of microalgal symbionts, the *Symbiodinium* dinoflagellates. This has concomitant effects in the homeostasis of the whole coral holobiont and its microbiome communities, yet the patterns of this unbalance are still not well defined. There are also pathogen driven disorders involving microbial agents responsible for significant declines in corals, especially those that induce tissue loss (white syndrome, WS). During the recovery from an intense bleaching episode that took place after the remarkably warm summer of 2014, which affected ˜80% of the reefs in Kane'ohe Bay, a devastating outbreak of WS also hit populations of *Montipora capitata*. Colonies were tagged in the field and subsampled at different time-points from the climax of the bleaching through the recovery for MISeq sequencing of the 16S V4, ITS-1 and ITS-2 regions. This presentation exposes our data tracing the populations of *Symbiodinium*, fungi, and bacteria in bleached and resistant corals following this incisive event, and contrasts these microbial community patterns with those associated to colonies with WS. The ability to adjust symbiotic communities in response to stress represents a key process for corals adaptation in a changing world. Still information is needed to understand the dynamics of microbial communities, and how they determine health, resistance and disturbance in coral reef ecosystems.

CONTRIBUTED PAPERS — Thursday, 08:15-10:00 – ***Vouvray***

Microbial control 5 - *Ben Raymond*

Controlling Invasive Crustacea

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Invasion biology studies invasive organisms and their ability to carry and transmit invasive diseases. Invasive diseases are becoming a commonly researched topic where scientists hope to understand diseases carried alongside, or left behind by, an invasion to develop functional biocontrol agents. Further understanding of invasive disease benefits biocontrol agent development but also aids in assessing risks to wildlife. In aquatic environments, 1054 invertebrates are acknowledged as invasive species across the globe. The greatest number of these belongs to the Crustacea (n=325). Crustaceans have been found to cause extensive damage to biodiversity, ecosystems and the environmental integrity of oceans and river systems. Their control is imperative to prevent ongoing destruction but is limited to physical and generalised chemical methods currently. Technologies show promise for future development in the fields of biological control, RNA interference and specific Cry toxins. Some of the world's worst invasive Crustaceans include the European shore crab *Carcinus maenas* (global invader) and the Killer Shrimp, *Dikerogammarus villosus* (European invader). Both have been attributed to large losses of biodiversity and require control. Both also harbour pathogens that could be adapted as biocontrol agents.

I present pathogens and parasites isolated from several invasive crustacean species that show promise as biocontrol agents. The screening efforts have identified several 'invasive pathogens' that may be harmful to aquatic ecosystems and their inhabitants. Crustacean control is lacking and research is crucial to forward the development of useable methods to control Crustacea in both environmental and aquaculture circumstances.

Molecular characterization of the plasmid-encoded Pir-like binary toxins isolated from shrimp suffering acute hepatopancreatic necrosis disease or early mortality syndrome (EMS/AHPND)

Kallaya Sritunyalucksana, Jiraporn Srisala, Suparat Taengchaiyaphum, Anuphap Prachumwat, Ornchuma Itsathitphaisarn, Timothy Flegel
Shrimp–Virus Interaction Laboratory, National Center for Genetic Engineering and Biotechnology, Bangkok, Thailand

Early Mortality Syndrome (EMS) also named Acute Hepatopancreatic Necrosis Disease (AH- PND) is considered as a new emerging shrimp disease that has been attacked to shrimp farms in Asia. Shrimp mortality occurred early after stocking of post larvae in shrimp cultivation ponds. The causal agent of EMS/AHPND was identified as a specific type of *Vibrio parahaemolyticus* (VPAHPND), which colonized the shrimp stomach and produced soluble toxins that entered the hepatopancreas to cause cell sloughing characteristic of AHPND. Recently, we have identified and characterized potential toxin(s) from VPAHPND isolates grown in broth cultures. Analysis of the active fraction revealed two major toxins of 16kDa (ToxA) and 50 kDa (ToxB). Mass spectrometry analysis followed by MASCOT analysis revealed that both proteins had similarity to hypothetical proteins of *V. parahaemolyticus* M0605 (GenBank accession no. JALL01000066.1) and similarity to known binary insecticidal toxins called '*Photorhabdus* insect related' proteins A and B (Pir-A and Pir-B), respectively, produced by the symbiotic, nematode bacterium *Photorhabdus luminescens*. In *in vivo* tests, it was shown that recombinant ToxA and ToxB were both required in a dose dependent manner to cause AHPND pathology, indicating further similarity to Pir-A and -B. Recently, the draft sequences of VPAHPND isolates from Thailand have been published. Sequence analysis confirms the presence of a homolog to the insecticidal *Photorhabdus* insect-related binary toxin Pir-AB located in a unique, previously unreported, large extrachromosomal plasmid.

Cancelled

DEBtox modelling of pathogen-mortality data over time; a novel toxicokinetic-toxicodynamic approach to derive dose effects

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Virulence of invertebrate pathogenic microorganisms is generally assessed using dose-mortality bioassays to generate response curves. Estimate LD50/LC50 summary statistics at a determined time point is calculated, often using Probit analysis. Lower effect dose estimates may also be derived e.g. LD10 values but these are difficult to measure and variability is considerable. Recently, we examined whether a process-based approach starting from the Dynamic Energy Budget theory (DEB) could be used. DEB theory is basically a simple set of rules for how organisms acquire and use mass and energy to maintain their life history from birth to death and is extensively used in ecotoxicology. The process-based approach has a significant advantage in that the effects for all concentrations and all the intermediate time points are used in the analysis. This allows prediction of effects under untested conditions e.g. over time points beyond those of the assay. In addition lower effect dose estimates can be made in a more robust way. Experimental data were obtained using the cabbage moth *Mamestra brassicae* as an exemplar Lepidoptera model system in which to test the application of DEB models to describe time-dependent dose-mortality responses. Bioassays were run with two pathogens and a comparator chemical, namely: the nucleopolyhedrovirus *Autographa californica* NPV; the bacterium *Bacillus thuringiensis* var. *kurstakii*; and the pesticide Spinosad. Here, we describe for the first time the use of DEBtox analysis to describe experimental data sets involving entompathogenic microorganisms.

A putative esterase is involved in toxicity of the mexican strain *Serratia entomophila* Mor4.1 towards larvae of *Phyllophaga* Spp (Coleoptera)

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The entomopathogenic bacteria *S. entomophila* strain Mor4.1 (*SeMor4.1*; *Enterobacteriaceae*) is active against larvae of the soil dwelling pest *Phyllophaga* spp (Coleoptera) by oral or injection bio-assays. The bacterium was isolated in Mexico from the haemocoel of a *Phyllophaga blanchardi* larva. Insecticidal activity against larvae of *Phyllophaga* spp and the lepidopterans *Manduca sexta* and *Spodoptera frugiperda* has been observed by injecting either, the bacteria or the cell free culture broths, suggesting that *Mor4.1* is able to produce and liberate to the media toxin-like factors with a wide spectrum of action showing activity at the level of the gut and/or at the hemocoel. To identify *SeMor4.1* toxic factors a fosmid *SeMor4.1* genome library was prepared in *Escherichia coli*. The clones were evaluated by injection bio-assays in larvae of *Phyllophaga blanchardi* and five insecticidal clones were selected. Injection bio-assays done with concentrated cell-free culture broths from D5 sub-clones have shown that two clones carrying DNA fragments about 2.5 Kb (clone D5.25) an 3 Kb (clone D5.3) presented high toxic activity, killing 90% and 60% of the *Phyllophaga* larvae evaluated after 24 h from the onset on bioassay. DNA sequence from the clone D5.25 shows that the clone codes for proteins with homologies to a putative Esterase and a major facilitator superfamily transporter from *S. marcescens.* Deletion mutagenesis of the clone D5.25 and analysis of decrements of insecticidal activity by injection bio-assays showed that the putative esterase is involved in insecticidal activity. The characterization of *SeMor4.1* toxins will help to evaluate their use for future biotechnological application for the management of insect pests.

Monitoring and expression analysis of *Pseudomonas* protegens CHA0 during colonization of Lepidoptera

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Some members of the genus *Pseudomonas* are known for their capacity to promote plant growth and for their efficacy in biological control of different plant pathogens. Besides plant diseases, insect pests are one of the major problems with great economic significance and, probably, the most challenging to control in agriculture. We recently discovered that some species of the fluorescent *Pseudomonas* group are capable of colonizing the gut of certain insect larvae after oral uptake and, subsequently colonize the hemocoel causing systemic infection and larval death. At present we are investigating the potential of these bacteria to be used against insect pests. In *Pseudomonas protegens* CHA0, insecticidal activity is mediated by the production of the Fit toxin. However, removing the *fit* gene did not completely eliminate the toxicity of this strain. This suggests that further virulence factors and mechanisms are involved in bacterial insect infection, which have yet to be elucidated. We are currently monitoring the proliferation of strain CHA0 in the insect gut and hemolymph during different stages of infection using strain- specific qPCR. In addition, the expression of genes involved in the production of the Fit toxin and putative additional virulence factors such as HCN and a cyclic lipopeptide are measured inside the insect. First results will be presented. The final aim is to perform an RNA-seq analysis to determine the genes the bacterium needs to switch between a root- and an insect-associated lifestyle. This knowledge will contribute to understanding *Pseudomonas* -insect interactions and to develop future *Pseudomonas* -based insect control strategies.

MICROSPORIDIA DIVISION SYMPOSIUM

Thursday, 14:00-16:00 - ***Bourgueil***

Host Pathogen interactions - *Susan Bjornson*

SYMPOSIUM. Thursday, 14:00 **212**

Inhibition of apoptosis is a universal mechanism for intracellular survival of microsporidia?

Yuliya Sokolova[1,2], Xavier Alvarez[3], Lisa Bowers[3], Elizabeth Didier[3]

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Intracellular parasites are known to develop mechanisms promoting or inhibiting cell death. Eukaryotic pathogens were studied less than prokaryotes in this respect, but protists of the genera *Theileria*, *Toxoplasma*, *Leishmania*, *Haplosporidium*, and *Perkinsus* all have been shown to inhibit apoptosis in the infected cells. Likely, this mechanism of intracellular survival is explored also by microsporidia (M). Indeed, widely distributed ability of M to induce cell hypertrophy and increase the lifespan of infected cells, suggest potential to hijack the host cell cycle control pathways. Inhibition of apoptosis was first presumed basing on *in vitro* studies of *Anncaliia algerae* (Scanlon et al., 1999). Later, it was straightforwardly demonstrated that *Encephalitozoon* spp. down regulated caspase-3 expression in Vero cells and inhibited p53 translocation to the nucleus (del Aguila et al., 2006). *Nosema ceranae* prevented ventricular epithelium cells from apoptosis, which was confirmed by TUNEL and IFA with caspase-3 antibodies (Higes et al.,2013). *N. bombycis* suppressed mitochondrial apoptotic pathway in the infected BmN cells (He at al., 2015). In our experiments *E. cuniculi* and *Vittaforma corneae* inhibited staurosporine- induced apoptosis in THP-1 cell line. Lower levels of TUNEL staining and caspase-3 activity in infected vs. non-uninfected macrophages corroborated with the data of microarray analysis: anti-apoptosis genes including BLC2 and TP53, were up-regulated in macrophages infected with M, while pro-apoptosis genes such as FADD, CASP3, CD40LG and others, were up-regulated in the macrophages incubated with dead, but not live, organisms. Molecular mechanisms that M use to regulate host apoptosis are yet to be identified.

SYMPOSIUM. Thursday, 14:30 **213**

Mosquito-Microsporidia Model Systems for Understanding Morphological and Phylogenetic Relationships

James Becnel

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Detailed studies on microsporidia can be extremely difficult due to the inability to easily manipulate the parasite and the host organism. Microsporidia in mosquitoes have proven to be excellent model systems because they have relatively short life cycles and are readily manipulated in the laboratory. Microsporidia are common in mosquitoes including a large clade of closely related genera and species with complex life cycles. Studies on this group of microsporidia in mosquitoes have provided many new details on the developmental cycles and phylogenetic relationships of microsporidia in general, as well as insights into their biological relationships with their hosts. These polymorphic microsporidia are characterized by complex life cycles involving multiple spore types responsible for horizontal and vertical transmission. They affect two generations of the mosquito and some involve an obligate intermediate host and alternations of haplokaryotic and diplokaryotic sequences. Polymorphic microsporidia are generally very host and tissue specific with complex developmental sequences comprised of unique stages and events. Details on key representatives of various genera will be presented including *Amblyospora*, *Parathelohania*, *Culicospora*, *Culicosporella*, *Edhazardia* and *Hyalinocysta*. The diversity of morphological and biological characters within and between species of these genera will be discussed with particular emphasis on the interpretation of phylogenetic relationships.

SYMPOSIUM. Thursday, 15:00 **214**

Pathogenicity, prevalence and intensity of a microsporidian infection by *Nosema fumiferanae* postvittana subsp. n. in the light brown apple moth, *Epiphyas postvittana*, in California

Julie Hopper[†1,2], Wei-Fone Huang[3,4], Leellen Solter[3], Nicholas Mills[1]

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We recently isolated a novel microsporidian pathogen from the exotic light brown apple moth, *Epiphyas postvittana* (Tortricidae), a potential pest of fruit crops and ornamentals in coastal California. We characterized the isolate and investigated its potential role in the biotic resistance of this exotic moth. Ultrastructure images and sequences from selected genetic markers confirmed this isolate to be closely related to *Nosema fumiferanae* (*Nosema fumiferanae postvittana* subsp. n.). We examined the latent period and pathogenicity of this microsporidium in the laboratory and determined its prevalence and intensity in five field locations using qPCR. The mean latent period for infections with 10^3 spores was 12.67 days, and when comparing healthy larvae to infected larvae, we found a highly significant reduction in juvenile survivorship, prolongation of

juvenile development time, a severe reduction in lifetime fecundity and a reduction in the intrinsic rate of increase. We detected *N. fumiferanae postvittana* in all field locations with a mean intensity of 226 spores and an overall prevalence of 5%, which did not appear to be influenced consistently by either host density or season. Although laboratory results demonstrated the potential for host suppression, field sampling indicated that prevalence and intensity were too low to solely account for the observed decline in population densities of *E. postvittana* in California. As *E. postvittana* is highly polyphagous (over 500 host plant species), we also investigated the role of plant quality on the fitness of healthy and infected *E. postvittana* larvae and discuss the relevance of tritrophic interactions to population dynamics in the field

SYMPOSIUM. Thursday, 15:30 **215**

Comparative genomics of microsporidia that infect marine organisms

Bryony Williams
Biosciences, College of Life and Environmental Sciences, University of Exeter, Devon, United Kingdom

The microsporidia are now becoming increasingly important aquacultural pathogens. Species in the family Enterocytozoonidae are unusual in terms of their host specificities: whilst most species infect aquatic vertebrates or crustaceans, one species, *Enterocytozoon bieneusi*, commonly infects humans. They are also peculiar in terms of their relationship with the host cell. Microsporidia typically live in the cytoplasm of the infected host cell, but some members of the Enterocytozoonidae sit tightly apressed to the host nuclei and others even exclusively inhabit the host nuclei in their intracellular stages. *E. bieneusi* has been previously shown to have an even more highly reduced metabolic repertoire than other microsporidia. A past genome survey of this organism showed that its glycolytic pathway has been lost, a surprising finding considering that in the microsporidia the components responsible for generation of ATP via oxidative phosphorylation were lost early in evolutionary history. We have sequenced and analysed genome data from new members the Enterocytozoonidae and have analysed patterns of loss of the glycolytic pathway in the microsporidia and carried out a comparative analysis of the transporter repertoire in intranuclear versus cytoplasmic pathogens with a view to finding molecular signatures of intracellular life.

CONTRIBUTED PAPERS — Thursday, 14:00-16:00 – ***Courteline***

Bacteria 4 - *Marianne Carey & Shuyuan Guo*

Contributed paper. Thursday, 14:00, **216**

How to eat a Crystal protein: Crystal protein Cry5B as a novel and powerful anti-infective for humans

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Soil-transmitted helminths (STHs), most notably, hookworms, whipworms, and *Ascaris*, are nematodes that infect more than 1.5 billion of the poorest people and are amongst the leading causes of morbidity worldwide. Only two classes of de-worming drugs (anthelmintics) are available for treatment, and only one is commonly used in mass drug administrations. New anthelmintics are urgently needed to overcome emerging resistance and to produce higher cure rates. Crystal (Cry) proteins, in particular Cry5B, made by *Bacillus thuringiensis* (Bt) are promising new candidates. Cry5B has excellent anthelmintic properties against many free-living and parasitic nematodes, including *in vivo* efficacy against multiple STH infections in rodents (*Heligomasmidoes polygyrus* and *Ancylostoma ceylanicum*) and in pigs (*Ascaris suum*). An enormous challenge for STHs, very different from most diseases worked on in the developing world, is the requirement that therapies be very cheap (the people infected are very poor and current drugs costs pennies a dose), massively scalable (over 4 billion people are at risk from infection), and have a long shelf life in harsh environments, that have high temperature and humidity and no cold chain. We will update our progress in several key areas. We will present new data on the *in vivo* activity of Cry5B against a major human parasite. We will also present data on the whether or not the immune system is required for Cry5B action *in vivo*. We will also present on our development efforts to produce a deployable version of Cry5B that is cheap, safe, scalable, and stable. These efforts are currently focused on bacterial engineering, expression, and formulation.

Contributed paper. Thursday, 14:15, **217**

A biochemical comparison of VIP3Ab1 and VIP3B insecticidal proteins

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Vegetative insecticidal proteins (VIPs) are soluble members of the *Bacillus thuringiensis* family of insecticidal proteins and share no sequence similarity to parasporal crystal (Cry) proteins. While the VIP family of proteins have been proven to be toxic to several orders of pests (Coleoptera, Hemiptera, and Lepidoptera), the VIP3 subfamily shows specificity for Lepidopteran insects. Importantly, VIP3 proteins have demonstrated different binding sites in Lepidopteran midgut relative to Cry proteins and thus represent a distinct mode of action for the development of transgenic crops. To date, 3 additional subsets of the VIP3 family have been identified; VIP3A, VIP3B, and VIP3C. Understanding the biochemical and insecticidal properties of these families will help determine their relative value for development of insect- resistant crops. In this study, we perform a biochemical comparison of VIP3Ab1 and a VIP3B protein that are known to have unique insecticidal spectra. We show that both proteins are tetrameric in solution and display differential sensitivity to gut juice proteolysis. Interestingly, both VIP3A and VIP3B proteins persist in tetrameric form before and after proteolysis. This observation questions the role of enzymatic processing in VIP3 proteins and suggests that this step may be a key determinant of insecticidal spectrum. Thus, unique biochemical characteristics of VIP3A and VIP3B proteins, such as processing and tetramerization, are likely to contribute to their differential spectrum, making them well suited for molecular stacks in transgenic plants.

Bio-polymer microencapsulations of *Bacillus thuringiensis* crystal preparations for improved longterm larvicidal activity

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Parasporal crystals synthesized by *Bacillus thuringiensis* (Bt) have been widely used as microbial pesticides because of their toxicity to specific insects in their larval stage. However, parasporal crystals can be influenced by different environmental stresses, such as high temperature, ultraviolet radiation, and desiccation. To enhance the resistance of parasporal crystals in the environment and to extend their activity, we have developed a new type of protection by making microcapsules of crystals (MC). The microcapsules are self-assembled by the alternate deposition (layer by layer) of low-cost chitosan and sodium alginate (or sodium carboxymethyl cellulose) on the surface of parasporal crystal. Crystal toxins (Cry 1Ac) are released from microcapsules above pH 9.0. Bioassays demonstrated that microencapsulated preparations had statistically equivalent larvicidal toxicity to the non encapsulated form. Interestingly, in microcapsules, crystals were protected to several environmental stresses, such as high temperature, UV radiation and desiccation. The results indicate that this kind of protection has a potential to enhance the efficacy of Bt in pest control. This is the first report of Bt crystal microencapsulation *in vitro*.

Enterocyte purge and rapid recovery as a novel reaction of the gut epithelium to toxin or xenobiotics exposure

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Host defense is not limited to immunity and encompasses the ability to endure and repair damages and to handle toxins and toxicants. We have found in the fly intestine a common response to *Serratia marcescens* hemolysin, a pore-forming toxin, and to xenobiotics such as caffeine, soft or heavy metals, or a strong oxidant. This phenomenon involves neither cell death nor the activation of classical stress response pathways. Exposure of enterocytes to hemolysin leads to the rapid controlled extrusion of the cytoplasm along with damaged organelles. This results in a thin intestinal epithelium that recovers its original shape in a few hours through regrowth of the enterocytes and not via the compensatory proliferation of intestinal stem cells. Epithelial thinning followed by rapid recovery of enterocyte shape after a hemolysin challenge is a process conserved from honeybees to humans. The recovery phase requires *CyclinJ*, an evolutionary conserved Cyclin of hitherto unknown function. CyclinJ is required for a rapid transcriptional response observed in the thin epithelium. A primary exposure to toxin or xenobiotics induces cross-protection against a further hemolysin challenge, which can be elicited by ectopically expressing *what else,* a *CyclinJ* -dependent gene that can fully rescue the *CycJ* mutant phenotype. Thus, *CyclinJ* plays a central role in this novel resilience mechanism that defends the intestine against occasional infections or intoxications.

Biomphalysin, a novel family of snail immune effectors with common features with bacterial pore-forming toxins

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In a previous study, we reported the horizontal acquisition from bacteria to the snail vector of schistosomiasis (*Biomphalaria glabrata*) of a *β*-pore forming toxin (*β*-PFT) named biomphalysin. This novel *β*-PFT displayed hemolytic and anti-schistosomal activities and shared structure feature homologies with aerolysin, a well-known bacterial virulence factor. The availability of the recently sequenced and assembled genome of *B. glabrata* allowed us to investigate the presence of additional aerolysin-like genes, leading to the identification of a total of 23 intron-less biomphalysin genes located into 22 different scaffolds. Comparative analyses of their deduced amino acid sequences revealed an important range of sequence identity while a high degree of structural homology is predicted. Contrasting patterns of tissue-specific expression of the different biomphalysin genes was observed, which suggest potential non-redundant functions. Interestingly, such pattern of horizontal acquisition and expansion of biomphalysin-like genes have already been observed in other animals, such as the tick *Ixodes* or the marine snail *Aplysia* but not in bivalve mollusks. This taxonomic distribution implies that some *β*-PFTs were probably acquired horizontally several times from bacteria to eumetazoa. These acquisitions were generally followed by gene amplification and diversification, allowing them to acquire different functions to become new weapons to fight against a high diversity of pathogens.

Toxicological and protein characterization of *Bacillus sphearicus* C3-41 strain from Karnataka, India

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A native virulent *Bacillus sphaericus* C3-41(CP 000 817) was isolated and identified from Rabbanahalli village of Yadgir district, Karnataka, India. The isolates from different geographical origins have different degree of toxicity and persistence in nature. Therefore, we made an attempt to characterize the pathogenicity and protein analysis of *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 30oC with 100 per cent 48 hr after the treatment.

Among the media tested forbioassay, 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 PAPERS — Thursday, 14:00-16:00 – ***Descartes***

Virus 7 - *Madoka Nakai & Gary Blissard*

Contributed paper. Thursday, 14:00, **223**

Comparative genomics of parasitoid wasps and what it tells on the evolution of symbiotic viruses

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Bracoviruses are symbiotic viruses stably associated with parasitoid wasps of the braconid family belonging to a monophyletic group, the microgastroid complex. They play a crucial role in the wasp life cycle as they produce bracovirus particles, which are injected into parasitized lepidopteran hosts during wasp oviposition and are necessary for successful development of wasp larvae within the host. Bracovirus particles package multiple dsDNA circles encoding virulence genes, while the genes used to produce the particles reside permanently in the wasp genome. The virulence genes are expressed by parasite host cells and produce factors inducing manipulation of host physiology including inhibition of lepidopteran immune defences. Each wasp species possess his own virus but they all derive from a unique event: the stable integration of a large DNA virus in the genome of their common ancestor. After the initial integration the virus has been chromosomally transmitted and has evolved differently depending on the wasp lineage. By comparing parasitic wasp genomes recently sequenced, we have analysed the dynamic of viral sequences evolution within the wasp genome, which differs between the genes involved in particles production in the wasp ovaries and those coding for virulence genes expressed in the parasitized host. Viruses associated with parasitic wasps are to date a unique example of endogenous viruses conferring a functional benefit to the host. Our comparative genomic analysis provides insights into how endogenous viruses evolve when they confer a benefice to their host.

Contributed paper. Thursday, 14:15, **224**

Permissiveness of lepidopteran hosts is linked to differential expression of bracovirus genes

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Polydnaviruses (PDV's) are DNA viruses associated with parasitoid wasps. *Microplitis demolitor* carries *M. demolitor* bracovirus (MdBV) and parasitizes the permissive host *Chrysodeixis includens*. In contrast, the related moth *Trichoplusia ni* is a nonpermissive host for *M. demolitor*. Many of the factors responsible for the permissiveness of *C. includens* are well studied, but why *T. ni* is a nonpermissive host remains unclear. Our data showed that *M. demolitor* successfully parasitize both hosts, but unlike *C. includens* wasp offspring failed to develop in *T. ni*. Segment abundance assays showed that viral DNAs persists in both hosts, but the deep sequencing data showed that virulence genes responsible for suppression of host immune defenses were expressed higher in *C. includens* than *T. ni*. Quantitative reverse transcription PCR assays confirmed the deep sequencing data. Several MdBV genes showed a differential pattern of expression in cell lines derived from these two lepidopteran hosts. Functional studies revealed that key MdBV virulence genes Glc1.8 and Egf1.0/Egf1.5 were sufficiently expressed to disable encapsulation and melanization in both *C. includens* and *T. ni.* Taken together, these data identify lower abundance of VIRAL gene products in defining host permissiveness for polydnavirus-carrying wasps.

Contributed paper. Thursday, 14:30, **225**

Latency-deficient recombinant and mutant Helicoverpa zea nudiviruses that cause enhanced pathology and sterility to their insect hosts.

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The Helicoverpa zea nudivirus 2 (a.k.a. gonad specific virus) is the only known sexually- transmitted insect. HzNV2 was isolated because it caused problems when rearing this moth. HzNV2 was then found to establish non-pathogenic latent infections in approximately 2/3 of infected insects rendering an agent of limited potential utility in biological control. Wu et al. (2010) showed that latency of HzNV1, a related virus isolated from H. zea cell cultures, is regulated by the persistence associate gene (*pag1*). To determine if the HzNV2 was similarly regulated, pag1 was replaced with yellow fluorescent protein (YFP). YFP-HzNV2 did not exhibit a latent phase in cell culture or in insects. The recombinant HzNV-2 also caused 100% of insects to become sterile. To produce virus mutants that lacked a latent phase, HzNV-2 infected Sf9 cells were chemically mutagenized and then plaque purified. Individual viral mutants were then injected into larvae and the effects on fecundity assessed at the adult stage. Three mutants were identified that caused sterility in essentially all infected *H. zea.* These mutants were characterized and shown to be sexually transmitted and reduce fecundity to a greater degree than the wild-type virus when female moths are infected. Surprisingly, when third instar larvae were infected with the YFP recombinant virus, mortality was observed which does not occur after injection of the wild type virus. This enhanced pathology could be explained as an outcome by the loss of latency in these viral recombinants. The results indicate that the recombinant and mutant viruses do not exhibit a latent phase and have enhanced pathology. The potential utility of such a sexually-transmitted mutant nudivirus to suppress Heliothine insects will be considered.

The postfusion 3D-structure of the Spodoptera exigua multiple nucleopolyhedrovirus envelope fusion protein F

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Baculoviruses utilize envelope fusion proteins (F or GP64) on the surface of budded virions for low-pH dependent membrane fusion to enter insect cells. Baculovirus F and GP64 proteins belong to Class I and Class III envelope fusion proteins, respectively. The baculovirus F protein may represent the archetype F protein, whereas GP64 has been acquired much later by (Group I) alphabaculoviruses. Cell entry via the F protein requires proteolytic cleavage of F into F1 and F2 in order to become fusogenic. To understand the function and mode of action of baculovirus F proteins the 3D-structure is important to complement the current biochemical and genetic studies. The 3D-structure of GP64 confirmed that it belongs to the Class III fusion proteins (Kadlec et al., 2008). Here we report the 3-D structure of the post-fusion ectodomain of baculovirus F protein (*Spodoptera exigua* nucleopolyhedrovirus). This protein lacking the transmembrane anchor domain and the C-terminal cytoplasmic tail, and containing a trypsin cleavage site downstream of the furin cleavage site was expressed in *Drosophila* S2 cells. Purified F ectodomains were deglycosylated with PNGase-F and treated with trypsin and acidic pH allowing conformational rearrangement into the postfusion trimeric state. The crystals were subjected to X-ray crystallography and diffracted to 2.7-3.4 °A resolution. This 3D-structure confirmed computational predictions that the baculovirus F protein adopts a Class I fusion protein fold and is homologous to the mammalian paramyxovirus F protein. The baculovirus F protein, from a DNA virus, possibly is the archetype F protein of vertebrate RNA virus F proteins. The results imply interesting evolutionary links between DNA and RNA viruses and their hosts.

A new system for studies of viral envelope protein trafficking in insect cells

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Trafficking of viral envelope proteins to the plasma membrane is critical for egress of most enveloped viruses. For many orally-acquired viruses of insects, progression to a systemic infection requires polarized trafficking of envelope proteins to basal membranes in the infected midgut cells, followed by viral egress from the basal membranes. Because the cellular trafficking pathways are not well defined, identification of the pathway proteins will be important for strategies to interrupt virus transmission, or to enhance beneficial viruses. To identify trafficking pathway proteins, we developed *Drosophila*-based systems for studies in cell lines and midgut cells. To examine general trafficking pathways in cell lines, we developed a baculovirus-based transduction system for transient expression of AcMNPV GP64 or VSV G protein. dsRNA-mediated RNAi is used to deplete candidate cellular proteins and to identify those required for transport of GP64 or VSV G to the plasma membrane. We first focused on Rab GTPases which are involved in vesicular trafficking of cellular proteins. To study polarized trafficking, we engineered *Drosophila* fly lines for midgut-specific expression of GP64 or VSV G, and found that they traffic to basal membranes of the gut epithelia. This provides evidence that each viral protein encodes information for polarized trafficking. Crosses of these lines with fly lines expressing dominant negative constructs of cellular trafficking proteins, permits screening and identification of cellular proteins required for polarized transport. Combined, these cell and fly-based systems permit identification of trafficking proteins and pathways important for success of insect-vectored and insect-pathogenic viruses.

Rescue of the entry of AcMNPV fusion-defective mutants by low-pH triggering: higher fusion activity is required for GP64-mediated entry into mammalian cells compared to insect cells?

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AcMNPV can transduce a variety of mammalian cells low efficiently, and a low-pH trigger significantly improves the entry efficiency of AcMNPV in mammalian cells. To explore the mechanism, a series of GP64s with mutations in D295/D301 localized within the central helix stalk were made. The expression and infection of the most mutants are similar to that of wild type (WT) GP64. However, syncytium formation assay showed that while D295E and -H slightly reduced fusion activity of GP64, the others (D301E, -H, D295/301H, D295/301E) showed dramatic reduction in fusogenicity, with only 3-10% of WT level remained, implying that D301 is a crucial residue for low-pH dependent conformational change of GP64. Mammalian cell transduction reveals a perfect correlation between the entry efficiency and fusion activity of the mutants. For example, the transduction rate of D295E and D301E is ˜80% and ˜9% of WT, as their fusion activity is ˜88% and ˜7% of WT, respectively. Interestingly, while the low fusion activity of D301E mutant could be improved with pH gradually decreasing, the transduction efficiency of D301E was efficiently rescued by low-pH triggering after virus binding (84% of WT at pH4.8). Our major findings are: 1) the AcMNPV mutants harboring low-fusogenic GP64, although infected insect cells as efficiently as WT virus, their ability to transduce mammalian cells were greatly impaired; 2) low-pH trigger significantly rescue the entry of the mutants in mammalian cells, probably via improving the fusogenicity of GP64s. We propose that higher fusogenicity of GP64 may be required for AcMNPV to enter mammalian cells than insect cells, and this may explain why low-pH triggered direct fusion as a high efficient route for the entry of AcMNPV in mammalian cells.

Extra genomic DNA elements found in an entomopoxvirus

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Entomopoxviruses (EPVs) possess a single linear double stranded DNA genome. The genome of Adoxophyes honmai entomopoxvirus (AHEV) was extracted from purified virus particles and sequenced fully. Apart from the 228,750 bp viral genome the sequence revealed the presence of two extra genomic DNA elements of 11,449 and 12,085 bp. These elements, predicted to encode 22 ORFs including a capsid protein, a cysteine protease

and genome-packaging ATPase, exhibit the genomic characteristics of Maveriks/Polintons or polintoviruses. Reminiscent of virophages, which are parasitic entity whose replication depend on a host virus, these elements possessed ORFs encoding replication proteins. However virophage-like particles were not detected inside either the occlusion bodies or virions of AHEV by transmission electron microscopy. AHEV was inoculated into *A. honmai* larvae and DNA replication was quantified by qPCR. The data showed that the replications of both the viral genome and the extra genomic elements were synchronized. Expression of all 22 putative ORFs in both of elements was detected by RT-PCR. RT-qPCR also revealed that expression level of DNA polymerase, capsid proteins and ATPase DNA packaging protein were nearly equivalent. Lastly, the extra genomic elements were found distributed in 3 geographical isolates.

CONTRIBUTED PAPERS — Thursday, 14:00-16:00 - *Vouvray*

Microbial Control 6 - *Mike Brownbridge*

Contributed paper. Thursday, 14:00, **230**

Entomopathogenic fungi for managing exotic and endemic pests in vegetable crops in California

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California produces several commercially important vegetable crops, which are infested by various invasive and endemic pests. Invasive Bagrada bug (*Bagrada hilaris*) is rapidly spreading in California and other states in the US, causing significant damage in cole crops and other hosts. Endemic tomato bug (*Cyrtopeltis modesta*) is emerging as a new pest in tomato and zucchini. Honeysuckle aphid (*Hyadaphis foeniculi*) and rice root aphid (*Rhopalosiphum rufiabdominale*) were recently found attacking celery and causing significant yield loss. Laboratory assays demonstrated the potential of *Beauveria bassiana*, *Isaria fumosorosea*, and *Metarhizium brunneum* in controlling Bagrada bug. Field studies in organic broccoli, zucchini, and celery against the Bagrada bug, the tomato bug, and root aphids, respectively showed that *B. bassiana* with azadirachtin can be an effective management option for the farmers.

Contributed paper. Thursday, 14:15, **231**

Experimental devices treated with *Metarhizium brunneum* and its extract for spotted-wing drosophila *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) control

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Spotted wing drosophila *Drosophila suzukii* has recently been found in many countries infesting blackberry, blueberry, and strawberry crops. It has also been observed occasionally attacking other soft-flesh fruit such as plums, plumcots, nectarines, and figs under suitable conditions. In the current study the potential of the entomopathogenic fungus *Metarhizium brunneum* (Petch.) and its extract in two experimental devices was evaluated for the control of *D. suzukii*. An experimental autoinoculation device impregnated with spores of the strain EAMa 01/58-Su was designed for a lure-and-infect strategy. Using this devise, *D. suzukii* adult mortality was 72.2%, and average survival time values of 3.6 days, respectively. There were no significant differences between red and black colors used to attract the *D. suzukii* adults. Active horizontal transmission from treated males to untreated females (24% mortality) was less effective than passive horizontal transmission from treated females to untreated males (72% mortality). Oviposition reduction from adult *D. suzukii* exposed to *M. brunneum*, recorded as pupae counts, was 81% compared with control. On the other hand, a lure-and-kill device was designed to dispense the crude extract from the EAMb 09/01-Su strain that previously has shown acute mortality of *D. suzukii*. This extract mixed with feeding attractant caused 95.0% of *D. suzukii* adult mortality. These results show the high potential of *M. brunneum* to be used in lure-and-infect and lure-and-kill strategies in an IPM program for *D. suzukii* control.

Contributed paper. Thursday, 14:30, **232**

Optimization of a coating process for the development of *Metarhizium*-formulations for control of soil dwelling pests

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The aim was the optimization of a millet based fungal preparation for application against soil dwelling pest larvae, produced by fluidized bed and coating process. For the first experiments the fungal *Metarhizium brunneum* strain JKI-BI-1339 (=Ma43, F52 or BIOPESCO5) was produced in a liquid medium described by Adamek (1963). In this medium this strain is producing mainly mycelium but as well submerged spores. After filtering over mull the biomass (mycelium and submerged spores) was adjusted to a defined concentration and was used for coating the millet in a fluid bed dryer. When the coated millet was incubated on water agar the fungus was only growing on 45 % of the millet kernels. When a thin layer of nutrients like malt or/and peptone was coated on the millet before fungal coating the fungus was growing of up to 80 % of the kernels and the number of produced conidia per kernel was doubled. To proof which fungal material is suitable for the coating process in the next set of experiments aerial conidia, mycelium or submerged spores were coated on millet with or without a thin layer of the different nutrients. The best result of nearly 100 % outgrowth was achieved with conidia independently of the addition of nutrients. For mycelium and submerged spores outgrowth rates of 75 % were achieved. By adding malt or a mixture of malt and peptone, the outgrowth was increased up to 95 %. By adding peptone alone the outgrowth was decreased and the number of contaminated kernels was enhanced. When the efficacy of the different *Metarhizium* -formulations was tested against *Tenebrio molitor* best results were achieved with the mycelial formulation followed by the submerged spore formulation. A positive effect of the nutrient layer was only seen for submerged spores.

Physiological mechanisms of synergy between pyrethroid insecticide and entomopathogenic fungus *Metarhizium robertsii* on nontarget aquatic model species *Daphnia magna*

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Indirect effects of chemical insecticides on aquatic invertebrates' mortality via suppression of resistance to pathogenic microorganisms are still poorly understood. Chemical toxicants can reduce non-target aquatic invertebrates' resistance to specific and non-specific pathogens. This situation can be actual both in agrocenosis and natural biocenosis after pest control when preparations come to aquatic system. In this study the combined influence of pyrethroid insecticide esfenvalerate and entomopathogenic fungi *Metarhizium robertsii* on cladoceran *Daphnia magna* has been examined under laboratory conditions. Sublethal concentrations of esfenvalerate led to increased mortality of Daphnia from fungus. Thus increased mortality due to mixed treatment was observed at 24 and 48 hours compared to the separate applications. An 10-50-fold increase of detoxification enzyme the glutathione-S-transferase (GST) activity was detected ($p< 0.01$) under combined treatment compared to the separate applications of fungus and esfenvalerate. At the same time, the application of esfenvalerate alone significantly decreased GST activity in daphnids ($p< 0.0001$). Sublethal concentrations of esfenvalerate caused an increase of stress hormone (dopamine) level ($p< 0.001$) in 12 hours of exposure. Application of *Metarhizium* also induced the dopamine concentration ($p< 0.01$) at 24 hours post infection irrespective of the esfenvalerate presence. Obtained results suggest that sublethal concentrations of pyrethroid insecticide esfenvalerate can inhibit the activity of the enzymes of Daphnia's detoxifying system and raise the level of dopamine, which leads to a synergistic effect when combined with entomopathogenic fungus.

Virulence of wild and transformed strains of *Metarhizium anisopliae* ICIPE30 against *Rhipicephalus appendiculatus* and *Amblyomma variegatum* ticks

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Entomopathogenic fungi are being perceived as attractive and promising alternatives to chemical acaricides. However, they have not yet met the expectation because of their slow speed of kill and thereby, the inability to compete with cheaper chemical pesticides. The present study evaluates the virulence of wild type (WT) and transformed (AaF1CA7) strains of *Metarhizium anisopliae* ICIPE30 against different developmental stages (larvae, nymphs and adults) of *Rhipicephalus appendiculatus* and *Amblyomma variegatum* ticks in the laboratory. The pathogenicity of the fungus was improved by engineering it to express *Androctonus australis* neurotoxin AaF1CA7. The transformed strain was virulent against larvae and nymphs of both tick species at most of the concentrations tested as compared to WT. For instance, the LC50 values of WT was 2.6 x 107 conidia ml-1 compared to 1.9 x 105 conidia ml-1 with the transformed strain in *R. appendiculatus* larvae. The LT50 values of the WT were significantly high and ranged between 7.6±0.7-21.2±9.8 days as compared to the transformed strain, which ranged between 3.9±0.3-10.0±1.2 days. Similar trends were observed with larvae and nymphs of A. variegatum. Adults of both species were less susceptible to fungal infection except at the highest concentration (1x109 conidia ml-1) where the transformed strain caused mortalities of 63.3 and 50.0% in R. appendiculatus and A. variegatum, respectively. These results contrast the ones from Anopheles arabiensis where no increase in virulence by the transformed strain was observed. Further study should investigate the underlying mechanisms.

Field evaluation of the entomopathogenic fungus *Metarhizium anisopliae* for the control of cotton aphid *Aphis gossypii* on okra crop

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Aphids are recognized as one of the most important insect pest of a variety of crops globally including vegetables. In Kenya, *Aphis gossypii* Glover is an economically important pest of okra. At present, okra growers rely almost exclusively on chemical insecticide application to combat aphids without adequate knowledge of their impact on human health, environment and non-targets. An isolate of *Metarhizium anisopliae* (Metsch.) Sorok. with high pathogenicity to *A. gossypii* was tested in field trials as a potential alternative for control of this aphid species on okra. Weekly applications of *M. anisopliae* wer compared with the chemical insecticide, Karate*Q*R ، for two cropping seasons. *M. anisopliae* was applied at the rate of 1×10e12 and 5×10e12 conidia per ha, and Karate was applied at the recommended rate of 17.5 g a.i. per ha. In all the trials, aphids' density was significantly lower in the fungal and chemical insecticide treated plots compared with the untreated control. The reduction in the aphid density was up to 88% in fungus treated plots relative to the control. Up to 64% mycosis was observed in aphids sampled from fungal treated plots. Marketable okra pod yield was significantly higher in the fungus (21,070 – 23,064 kg/ha) and Karate (22,504 kg/ha) treatments compared to the control (17,279 kg/ha) during the first cropping seasons. However, no significant difference was observed on marketable pod yields among the fungus and Karate treatment during the second cropping season. The results demonstrate the prospect of managing *A. gossypii* with *M. anisopliae* in okra agroecosystem.

Non-target effects of *Metarhizium brunneum* on microbial communities assessed in pot and field trials to control *Agriotes* spp

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Biological and chemical plant protection treatments are well accepted for pest control, however, the impact particularly of biological control agents on soil microbial communities has been poorly investigated. Potential effects of biocontrol treatments on soil microbial communities were assessed in a pot and a field trial to test efficacy of the entomopathogenic fungus *Metarhizium brunneum* for control of *Agriotes* spp. larvae in potato production. Treatments included different formulations of *M. brunneum* (fungus colonized barley kernels (FCBK), capsules, granules and spore

powder), garlic capsules, the insecticide clothianidin, barley kernels (BK) and empty capsules. Soil samples were collected in both trials before, 7 and 15 weeks after application. DNA was extracted and PCRs targeting fungi or prokaryota were performed. Amplicons were sequenced using the Illumina MiSeq platform. Fungal applications led to a 10 to 100 fold increased *Metarhizium* spp. abundance in field and pot samples, respectively. Fungus treatments in the pots resulted in a significant reduction of *A. obscurus* larvae and the FCBK treatment resulted in 90 % undamaged potatoes compared to 20 % in untreated pots. No damage reduction was achieved in the field. In the pot trial prokaryotic communities were affected by garlic and fungal communities reacted primarily to FCBK and BK. Treatment effects were in the same range as time effects detected in controls during the course of the pot trial. In the field neither the fungal nor the prokaryotic communities were affected. The study revealed that BCA applications can have temporal effects on microbial communities in pot trials, which however, were not detectable in the field.

Contributed paper. Thursday, 15:45, **237**

Aprehend™ for bed bug control – the biological advantage

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A series of bioassays were conducted on the human bed bug *Cimex lectularius*, to evaluate the efficacy of Aprehend™, a *Beauveria bassiana* based, residual insecticide against bed bugs. Unlike competing chemical products, Aprehend™ is comprised of fungal conidia, and does not require long-term exposure to be effective. Furthermore, bed bugs exposed to Aprehen™ transport the fungal conidia back to their harborages, resulting in additional mortality due to horizontal transmission. These aspects in addition to the three-month residual activity following spray application of Aprehen™ make this biopesticide product one of the most promising new technologies for bed bug control. Here we present the data that support these claims and discuss how use of Aprehend™ fits within an overall bed bug IPM strategy.

POSTERS

POSTER SESSION Wednesday, 10:30-13:30 – ***Agnès Sorel***

Bacteria division

Poster. **BA-1**

A novel protein active from a *Pseudomonas* strain with unique mode of action against western corn rootworm, *Diaborica virgifera virgifera* (LeConte)

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Western Corn Rootworm (WCR; *Diabrotica virgifera virgifera)* is a highly damaging pest in the major corn production area of North America, with the potential to cost U.S. farmers as much as $2 billion yield loss annually if not controlled. Transgenic crops expressing various *Bacillus thuringiensis* -derived insecticidal Cry protein genes have been on the market for over 15 years providing significant value to growers and positive impact on the environment due to the reduced need for chemical insecticides. Nevertheless, in the last few years increased resistance to Cry3-based transgenic trait products has been reported in the literature in WCR, highlighting the need for the discovery of alternative actives that can complement or substitute for Cry toxins. Here we report the discovery of a new insecticidal protein from a *Pseudomonas* strain displaying strong activity against WCR. Corn plants expressing this protein exhibit high resistance to WCR damage. Both biochemical and bioassay data indicate no cross resistance from laboratory generated resistant colonies of events expressing either of commercial proteins Cry3Aa or Cry34/Cry35. These results illustrate the potential of non-Bt sources of novel actives that may be important to the development of effective insect control traits to benefit growers in the future.

Poster. **BA-2**

Alkaline phosphatases are involved in the response of mosquito larvae to intoxication with Bti Cry toxins

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Bacillus thuringiensis subsp. *israelensis* (Bti) is a natural pathogen of dipterans widely used as a biological insecticide for mosquito control. In order to characterize the response of mosquitoes to intoxication with Bti, the transcriptome profile of Bti-exposed susceptible *Aedes aegypti* larvae was analyzed using Illumina RNA-seq. Gene expression of 11 alkaline phosphatases (ALPs) was further investigated by RT-qPCR and ALP activity was measured in the susceptible strain and in four strains resistant to a single Bti Cry toxin or to Bti. These strains were unexposed or exposed to their toxin of selection. Although all resistant strains constitutively exhibited a higher level of transcription of ALP genes than the susceptible strain, they showed a lower total ALP activity. The intoxication with different individual Cry toxins triggered a global pattern of ALP gene under-transcription in all the one-toxin resistant strains but involving different specific sets of ALPs in each resistant phenotype. Most of the ALPs involved are not known Cry-binding proteins. RNA interference experiment demonstrated that reducing ALP expression conferred increased survival of larvae exposed to Cry4Aa, confirming the involvement of ALP in Cry4Aa toxicity.

Poster. **BA-3-STU**

Aquaporins contribute to water influx into Sf9 cells intoxicated by *Bacillus thuringiensis* Cry toxin

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Bacillus thuringiensis Cry toxin disrupts ion balance of insect midgut epithelial cells and induces osmotic swelling and necrotic cell death. Water influx into cytoplasm induced by Cry toxin intoxication has been regarded to occur following influx of small solutes such as cation through the toxin pore that generates increase of cytoplasmic osmotic pressure. However, the route(s) of water influx and the extent to which water influx contributes to cell death were obscure. In the present study, we investigated the involvement of aquaporins (AQPs), membrane proteins responsible for water transport, in water influx caused by Cry toxins. Water influx into Sf9 cells exogenously expressing a Cry1A toxins receptor was inhibited by methylmethanethiosulfonate (MMTS), which is known as an AQP inhibitor. In this assay, decrease in water influx by AQP inhibition correlated with decrease in release of lactose dehydrogenase (LDH), a typical signature of necrotic cells. Na+ ion influx into cytoplasm caused by Cry toxin was observed under MMTS treatment, suggesting that MMTS did not interfere pore formation of Cry1Aa toxin on the cell membrane. Overexpressions of *Bombyx mori* AQPs in Sf9 cells caused the increase of speed of water influx in response to Cry1C intoxication, suggesting that the expression levels of AQPs are responsible for susceptibility to Cry toxin. Our results first demonstrated that AQPs are main routes of water influx caused by Cry toxin and that water influx via AQPs is a direct determinant of cell death by a bacterial pore-forming toxin.

Poster. **BA-4**

Association of cry genes from *Bacillus thurigiensis* with mortality in *Spodoptera frugiperda*

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Bacillus thuringiensis (Bt) is a gram positive bacteria that produces crystalline inclusions toxic to the Lepidoptera, Coleoptera and Diptera orders, besides mites and nematodes. Such inclusions are proteins produced by Cry genes. Fall armyworm, *Spodoptera frugiperda* (J. E. Smith), is one of the most important insect pest in Brazil. In maize this pest can reduce crop yield up to 35%. It is extremely important to developing effective tactics to control this pest. The objective of this work was to associate Bt genes of a collection of Bt strains to the toxicity against *S. frugiperda*. Bioassays showed that out of 94 Bt strains 10 showed mortality below 40%, 20 between 40 to 80% and 74 above 80% mortality, and 35 out of 74 showed a 100%. PCR products based on 26 specific primers for *cry* genes showed that there is a strong correlation of cry1D, cry1I and cry1F and mortality of *S. frugiperda*, and on average there are 5 cry genes/Bt strain. Some Bt strains had only one gene, some 12 genes and 1 strain showed 20 genes. The cry1D was the most frequent gene among the Bt strains. The less frequent one was cry9D. Loss of larval weight was visualized in many Bt strains. PCR products bands were purified from agarose gel and sequence showed non-specific amplification for some cry primers. These primers are under review. Isolation of complete coding sequence of some of these genes and expression in heterologous system such as *E. coli* is under way.

Poster. **BA-5 STU**

Biological control of *Hypsipyla grandella* Zeller (Lepidoptera: Pyralidae) with the systemic use of *Bacillus thuringiensis* Berliner on mahogany seedlings (*Swietenia macrophylla* king)

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Hypsipyla grandella Zeller, popularly known as mahogany shootborer, is the major pest of mahogany, limiting the establishment of commercial planting of the species in Brazil. Based on this, this study aimed to evaluate the use of *Bt* in mahogany plants for the control of *H. grandella*. For this, five strains used were chosen based on preliminary experiments, where all caused 100% of mortality in newly hatched larvae when mixed with mahogany seeds. In order to check the systemic effect in mahogany seedlings, the bacteria were mixed with autoclaves distilled water and the solution was inoculated into the soil at a concentration of 108 spores per mL for each treatment. The witness consisted of distilled water autoclaved without bacteria. Each treatment was repeated with six plants, and in each seedling were placed three eggs of *H. grandella* 48 hours old. Therefore, we used 36 seedlings, arranged in a completely randomized design, with 108 eggs of *H. grandella*. After 30 days, the parameters evaluated were: presence or absence of gum, web and excrement, size of the gallery formed by the insect and the number of live caterpillars / dead inside the plant. As a result, the plants treated with the streain S1905 showed an attack decelerated compared with the control, with little apparent damage and symptoms, characterized by little exudate, gum, excrement and warp by the insect. The untreated plants with *Bt* proved completely attacked, with up to three caterpillars inside, in addition to having the largest galleries (up to 15.7 cm long). Despite being a preliminary study, the results open a follow untapped in the forest area and that in the future can become one of the main methods for the controle of insect pests, especially with the use of systemic microorganisms.

Poster. **BA-6 STU**

Biomphalysin, a bacterial *β*-PFT family in the schistosomiasis vector snail, *Biomphalaria glabrata*

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Aerolysins are virulence factors belonging to the *β*-pore-forming-toxin (*β* -PFT) superfamily that are widely distributed in bacteria. Moreover, several *β*-PFTs have also been described in eukaryotic organisms acquiered by a horizontal gene transfer across kingdom. In the schistosomiasis vector snail *Biomphalaria glabrata*, using an interactom approach, we have identified a novel *β*-PFT displaying hemolytic and anti-schistosomal activities and sharing structures features with aerolysin. A phylogenetic approach in the snail genome investigation reveals an expansion gene of this multigenic family leading to the identification of a total of 23 biomphalysins in the Brazil strain but huge diversity seems appear in others snail strains available in our lab. Analysis of their expression highlighted various patterns of tissue-specific distribution, suggesting non-redundant functions. Considering the anti-schistosomal role of the first Biomphalysin identified, expression of all Biomphalysin genes was measured after snail exposure to different intruders This revealed that gene expression of some but not all biomphalysins was induced in parasite-exposed snails. Altogether, our data suggests that ”under pressure” a gene acquisition followed by an expansion lead to the acquisition of potential new functions and could constitute a new immune weapon against multiple and diversified *Biomphalaria* intruders.

Poster. **BA-7**

Cadherins are Cry5B Toxin Receptor in *Caenorhabditis elegans* and Play a Sequential Role with the Glycolipid Receptor

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The animal and plant parasitic-nematodes caused a worldwide devastating impact on people lives and a great damage to agricultural crops, receptivity. Several Cry toxins produced by *Bacillus thuringiensis* have potential to develop as safe, effective and affordable active molecules for nematode control. However, the action model of Cry toxin in nematode has yet poorly understood. In this study, we used *Caenorhabditis elegans* as model to study the mechanism of nematicidal Cry5B target nematode. We proved that nematode-specific cadherins CDH-7 and CDH-8 are function receptors for Cry5B, provide evidence that: cadherin mutant *cdh-7(RB685)* and *cdh-8(RB815)* showed significant resistance to Cry5B; CDH-7 and CDH-8 fragments specific bound to Cry5B and promoted Cry5B oligomerization. Additional, we showed that CDH-7 and CDH-8 act synergistically in responding to Cry5B, because the *cdh-7* and *cdh-8* double mutants showed more resistance than single mutants, and both lost toxin binding ability *in vivo*. Furthermore, we demonstrated cadherins were upstream receptors of glycolipid, the evidence includes: lacking of CDH-7 and CDH-8 did not affect Cry5B uptake into intestinal cells; CDH-7 and CDH-8 play more important role in toxin binding *in vivo* than Glycolipid; and reduction of *bre-5* function in cadherin mutants resulted to significant higher resistance than cadherin mutants. Our results demonstrated that nematode-specific

cadherins act as functional receptors for nematicidal Cry toxin, and provided evidence that pore-forming and glycolipid receptors play a role sequentially for Cry toxins.

Poster. **BA-8**

Characteristics of an entomopathogenic bacterium, *Xenorhabdus hominickii* ANU1 and its pathogenicity against two lepidopteran pests

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Two genera of entomopathogenic nematodes, *Steinernema* and *Heterorhabditis*, are mutualistically associated with their symbiotic bacteria in gut. This study reports on an entomopathogenic nematode isolate and its symbiotic bacterium, which were collected in Andong, Korea. Dead insect infected by nematode showed a typical cadaver symptom with a brown color, out of which thousands of infective juveniles emerged. The nematode isolate was identified as *Steinernema monticolum* by morphological characters and 16S ribosomal RNA (16S rRNA) sequence. Also, symbiotic bacteria from *S. monticolum* were isolated from the nematode-infected hemolymph of the fifth instar larvae of beet armyworm*, Spodoptera exigua*. Biolog bacterial identification system, several biochemical characters, and 16S rRNA sequence were identical to *Xenorhabdus hominickii*. The infective juvenile of nematodes and its symbiotic bacterium showed potent pathogenicities to two lepidopteran pests, *S. exigua* and *Plutella xylostella* by topical treatment and intra-hemocoelic injection, respectively. In whole genome study on bacteria by PacBio, the *X. hominickii* ANU1 genome assembled *de novo* by FALCON. The whole genome sequence comprises 4,522,699 bp with GC content of 43.4% and annotation revealed a total of 4,531 genes (4,199 CDs). The genome has 22 rRNAs and 87 tRNAs.

Poster. **BA-9**

Characterization of a *Wolbachia* strain native from Argentina for potential application as mosquito control agent

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Wolbachia pipientis is an endosymbiotic bacterium of the Ricketttsiaceae family that naturally infects the mosquito *Culex pipiens*, and affects insect reproduction and pathogen transmission. Cytoplasmic incompatibility is defined as embryonic lethality caused by crosses between infected males and uninfected females, or between males and females carrying incompatible *Wolbachia* sp. strains. Our aim is to report the preliminary characterization of a native *Wolbachia* strain from *Cx. pipiens* mosquito of Argentina in order to evaluate its potential as a vector control agent. We carried out the molecular identification of *Wolbachia* strains by PCR using primers for the amplification of a specific *Wolbachia* surface protein in different mosquito populations all around Mar del Plata city; one of the mosquito populations was the carrier of a *Wolbachia* strain highly similar to an incompatibility strain *w* Pip previously identified from *Cx. quinquefasciatus*. To confirm the strain ability to induce cytoplasmic incompatibility we generated a *Cx. pipiens* colony from a native mosquito population. The wildtype (Wsp+) colony was previously cured of its native *Wolbachia* infection by tetracycline treatment of adult females and males to obtain cured mosquito line (Wsp-). A 100 % of cytoplasmic incompatibility was observed in experimental crosses between Wsp+ males and Wsp- females which demonstrates that this strain is a good candidate for a potential application of the incompatible insect technique. Future work will be needed to determine its ability to interfere in the human pathogen transmission in *Cx. pipiens* and the possibility of artificially transfect to novel mosquito hosts. Supported by PICT 2013-0431, PIP 112 20110100963, 15/E692 - EXA742/15

Poster. **BA-10 STU**
Cancelled

Poster. **BA-11 STU**

Dam overexpression impacts motility and virulence of the entomopathogenic bacteria, *Photorhabdus luminescens* TT01

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Bacterial DNA methylation is known to play a role in gene expression (Casadesu´s et al., 2006). Dam is the most described DNA-methyltransferase and is widespread in gamma-proteobacteria. Dam DNA methylation is involved in pathogenicity of several bacteria. *Photorhabdus luminescens* is an entomopathogenic bacterium symbiotically associated with nematodes of the genus *Heterorhabditis*. The nemato-bacterial complex is able to kill insect larvae such as *lepidoptera* by bacterial septicemia (Nielsen-LeRoux et al., 2012). We identified a Dam ortholog in *P. luminescens* genome and we showed that *P. luminescens dam* gene is functional by cloning it on a plasmid in an *E. coli* Dam mutant. After overexpression of *dam* in *P. luminescens,* a decrease of about 50% in motility ($p < 0.02$) was observed. In addition, after injection of 1000 CFU in larvae of *Spodoptera littoralis,* the Dam overexpressing strain showed a delayed virulence compared to that of the control strain (harboring an empty plasmid). In contrast, no difference in growth ability was observed *in vitro* between the two recombinant strains. These results enhance our knowledge about Dam methylation and strengthen the hypothesis that Dam plays a major role for gene expression in proteobacteria. To go further, we plan to identify all DNA methylations in the genome of the two strains using Pacbio sequencing.

Poster. **BA-12 STU**

Detection and characterization of Parasporin proteins in *Bacillus thuringiensis*

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The Gram-positive bacteria *Bacillus thuringiensis* (Bt) is widely known for its importance on biological control, due to their ability to produce crystalline inclusions (proteins Cry e Cyt), active against a broad range of insects. A new activity was reported for crystals without insecticide activity, the cytotoxic against human cancer cells. These cytotoxic proteins are known as Parasporins (PS), are not hemolytic and have different structure from the Cry and Cyt proteins. The Embrapa Genetic Resources and Biotechnology has a collection of *Bacillus* spp. with approximately

2500 isolates. This work aims to identify, among the strains of this collection, genes of Parasporin families through different molecular markers, analyze the protein profile of strains, the possible production of β-exotoxins and evaluate its toxicity to tumor cells line. Two hundred and sixty-nine strains were selected randomly on the bacteria bank, and were screened with specific primers to identify the different Parasporin genes. Thirty-five strains showed amplification patterns, of which, 30 isolates showed amplification to the *parasporin 1* gene, and other 5 isolates to *parasporin 3* gene. The protein profile suggested that the secreted proteins by some strains has the expected size to the group of Parasporin. None of the positive strains identified by PCR showed β-exotoxins production. The protein of the tested strains didn't show toxicity against tumor lines DU-145 and HeLa, however PS1 from the strain S1338 showed specific toxicity against breast cancer cells MCF-7, changing their morphology after 24 hours of treatment with active toxin, suggesting that PS1 from S1338 strain has unique characteristics and specificity against MCF-7 cell line.

Poster. **BA-13 STU**

Dual action of *Bacillus thuringiensis* in the vegetative development of cotton (*Gossypium hirsutum* L.) and the control of *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

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4 Instituto Mato-Grossense do Algodão, Brazil

Bacillus thuringiensis (Bt) is used worldwide as a tool for control of lepidopteran pests. Recent studies have demonstrated the possibility of its use as endophytic organism, controlling insects and promoting plant growth. To learn more about these effects, strains of Bt toxic to insects of the Order Lepidoptera were inoculated both onto seeds and into cotton plants and were assessed for effectiveness of control of *Spodoptera frugiperda* and effect on promoting plant growth. It was observed that Bt influenced the height, dry weight yield and number of leaves on cotton plants and the development of the insect. Thus, it was possible to select a strain, a cultivar and inoculation method, suggesting that there is a close relationship between Bt strain and variety of cotton. This leads to the conclusion that Bt endophytically colonizes plants, acting simultaneously in plant growth promotion and potentially in insect control.

Poster. **BA-14**

Evidences for cross-order activity of binary Vip proteins

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The flatheaded borers, *Capnodis tenebrionis*, are major pests of stone fruit trees; larvae feed inside the roots, making difficult to control them. Implementation of transgenic trees expressing a toxic compound could help in pest management strategies. Cry and Vip proteins of *Bacillus thuringiensis* (Bt) are well known as environment-friendly insecticides that have been used in different commercial transgenic crops. In fact, several binary toxins Vip1/Vip2 have been described toxic to coleopteran species. Analysis of 40 Bt isolates provided 19 strains toxic to this insect species, and confirmed by dose response-bioassays of the 6 with the highest toxicity. In the present investigation, occurrence of the genes encoding Vip1 and Vip2 has been analyzed by PCR fragment amplification. Two strains (U13 and U16) displayed *vip1* and *vip2* fragments with 99% identity. Multiple pairwise alignment and Neighbour-Joining analysis with reported proteins discovered that they were grouped with Vip1Ac and Vip2Ae. These proteins were reported in a strain toxic to *Aphis gossypii* (Homoptera), but not for Coleoptera (*Tenebrio molitor* and *Holotrichia oblita*). The occurrence of the same gene combination in analyzed strains suggest that this binary protein could have cross-order activity (Homoptera-Coleoptera). Evidences about toxic activity to more than one insect order has been described for Cry proteins, but it is the first time that it can be pointed to any of the Vip1/Vip2 proteins combination. These preliminary experiments will be pursued to determine the toxicity to *C. tenebrionis* and the magnitude of cross order activity.

Poster. **BA-15 STU**

Evolution of *Photorhabdus* Virulence Cassettes

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Photorhabdus bacteria harbour a vast array of potent toxins. Acute lethality against the insect host via use of these toxins is vital to symbiosis with *Heterorhabditid* nematodes. The *Photorhabdus Virulence Cassettes* are one such mechanism. Each strain harbours 5-6 distinct27kb "PVC" operons, of ~18 genes. These encode a 'nanosyringe'-like complex, that translocates genetically coupled effectors to target cell interiors. PVC operons share gross structural similarity, resembling bacterial tailocins, but the coupled effectors are extremely diverse. PVC operons have the hallmarks of horizontally acquisition, and diversity among different homologs suggests they are prone to recombination. Bioinformatic analysis indicates that many PVC operons are linked to remnants of insertion elements and in some cases have the configuration of composite transposons. Through ClonalFrame analysis and basic sequence statistics, we estimate recombinatory breakpoints within the operons, and draw conclusions on the microevolution of PVCs. We also infer the degree to which paralogy within an operon is driving ORFs to fixation/removal. This provides insight into the effect of gene copy on the assembly stoichiometry of the PVCs, and the redundancy within the operons. With comparative HMM profiling, as well as *ab initio* structural simulations, we characterised all CDSs within the operons. We correlate the relationship between amino acid diversity and the predicted protein structures of the various PVC ORFs. In particular, we explored diversity in the 13th PVC ORF, which appears to be a chimeric bacteriophage fibre-like gene, and is likely to be responsible for targeting of specific cell types.

Poster. **BA-16**

Genomic and phenotypic analysis of *Bacillus thuringiensis* cry- exposed for *in vivo* experimental evolution in *Galleria mellonella*

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The exposition of a pathogenic bacterium to a host during a serial passage experiment (SPE) may facilitate the apparition and posterior fixation of mutations that favour its growth and multiplication in the host environment. Here we describe the results obtained for a SPE of a Bt407 Cry- strain (streptomycin resistant) (this parental strain was entirely re-sequenced by Roche 454 Genome Sequencer FLX) prior to the study in the host. An infection protocol was established where *G. mellonela* larvae were forced fed with spores of the Bt407 Cry- strain, then spores collected from dead larvae where used for to re-infect *G. mellonella* individuals in a next passage. The experiment lasted for 20 passages and with 9 lines in parallel. The genetic changes, which happened during the experimental evolution, were monitored by sequencing of the evolved populations at passages 5, 10, 15 and 20. In parallel, to study fitness, competitions assays were performed between evolved strains against the non-evolved initial populations using fluorescent markers. Finally the response of the evolved bacteria to nisin, an antimicrobial peptide used as a food preservative, was also compared with the response of the initial parental bacteria and with populations evolved *in vitro.*

Poster. **BA-17 STU**

Mechanisms involved in the acquistion of host iron ferritin by the opportunistic insect pathogen *Bacillus cereus*

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Iron acquisiton is essential for pathogenic bacteria. In hosts, the iron is not free: it is bound to proteins like transferrin or ferritin, used for storage and homeostasis, or included inside hemoproteins. In order to capture this iron, bacteria develop several systems including secreted high affinity iron binding molecules (siderophore) or surface proteins. In *Bacillus cereus* (opportunistic pathogen for insect and human, closely related to *B. thuringiensis)*, we have demonstrated that both the surface protein IlsA and the siderophore bacillibactin are involved in iron acquisiton from exogenous Mammalian ferritin, and are important for infection (Segond *et al*, 2014, Plos Pathogen). In this project, we aim i) to assess the importance of ferritin sources in iron acquisition efficiency of the IlsA and bacillibactin systems. We will focus on insect ferritin, in order to test the hypothesis that IlsA is better adapted to an invertebrate ferritin rather than vertebrate ferritin. Next, we'll investigate ii) the importance of siderophore binding protein FeuA in virulence of *Bacillus cereus* during infection of *Galleria mellonella*, used as insect model. Finally, iii) *in vivo* tests will be run to determine a time-related and/or tissue specific expression of bacillibactin and IlsA in the insect, by using reporter genes and microscopy observations. This PhD project will both provide molecular and mechanistic insights of *B. cereus* iron uptake during host infection, and new fallouts about possible bioavailability of ferritin iron from insect based powders for feed and food.

Poster. **BA-18**

Mosquitocidal activity of non-3-domain Cry type 33-kDa protein from *Bacillus thuringiensis* isolated in Japan

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Novel *Bacillus thuringiensis* strain, isolated from grove soil in Japan, produces a mosquitocidal inclusion body called crystal consisting of several Cry proteins during sporulation phase. Genome analyze using a next-generation sequencer revealed that this *Bt* strain has 13 *cry* genes. Structural analysis of amino acid sequences deduced from nucleotide sequences, twelve of 13 Crys have high similarities with Cry32 protein group, and could take the structure of 3-domains type. The last one of 13 Crys, Cytox, was 33 kDa, and this protein belonged to Crys with cytocidal activity, which exhibits structural similarity to the beta pore-forming toxins such as lysenin and aerolysin. *cytox* ORF was cloned into a *Bt* expression vector carrying *cyt1A* promoter and *cry4A* terminator, and was introduced into *Bt*. *kurustaki* CRY(-)B, a crystal non-production strain, and was cultured in sporulation medium. After harvesting by centrifugation, the cells were lysed by sonication. Cry protein was purified by NaBr gradient centrifugation method, and was used for mosquitocidal assay against *Ae. aegypti* larva and *Cx*. *pipiens* larva. Cytox showed high mosquitocidal activity whereas proteins of Cry32 group hardly showed activity. Cytox has almost none of the amino acid sequence similarity with Cry taking the structure of 3-domains, the insecticidal mechanism is expected to be different.

Poster. **BA-19**

Multifaceted aspects of insect pathogenic and commensal bacteria in insect based food and feed

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Like other organisms insects are associated with bacteria as commensals, symbionts or as pathogens. It seems obvious that aspects related to the role and impact of these microbes in insect based food, from rearing, during processing to storage and finally in the host, need to be explored at various levels. In the Micalis Institute (www.micalis.fr) we are working with several topics related to microbial food safety. Both to elucidate bacterial pathogenesis in insects, for instance with *Bacillus thuringiensis* (1,2,3) bacteria as probiotics (4) and bacteria in food conservation (5) the latter two are mainly belonging to species of lactic acid bacteria. Then although one of the interesting points with insects are the presumed low risk of transfer of pathogens from insect to vertebrates, there is a need for investigating the microbiota associated with the chosen insect's sources to evaluate benefice and risk. The poster will mainly highlight such aspects but will also raise ideas related to the potential of bacterial entomo-pathogens as enzyme sources for industrial biotransformation of insect based products. The aim of the presentation and hopefully the discussion is to set up research topics related to insect based food safety in order to include them in new collaborative projects.

Poster. **BA-20**
Cancelled

Poster. **BA-21**

New entomopathogenic bacterial strains from *Galleria mellonella* larvae infected with EPNs

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The knowledge on insect pathogenic bacteria with potential for pest management is significantly increased during the last decades. Whilst the spore-former *Bacillus thuringiensis* (*Bt)* still remains the most studied and commercially-exploited species, the discovery of new entomopathogenic bacterial species and isolates against novel targets is expected in the near future. In this study, major bacteria developing on *Galleria mellonella* larvae infected with recently collected entomopathogenic nematodes (EPNs) belonging to *Steinernema and Heterorhabditis* genera, were isolated and their pathogenicity was assessed against different insect pests of agricultural, forest and medical-veterinary importance, *including Lepidoptera (Lymantria dispar and Malacosoma neustria) and Diptera (Musca domestica and Ceratitis capitata).* This led to the identification of new entomopathogenic bacterial isolates belonging to different species including *Alcaligenes aquatilis*, *Alcaligenes faecalis*, *Enterococcus mundtii*, *Pseudomonas protegens*, *Serratia maltophilia*, *Serratia marcescens* and *Stenotrophomonas nematodiphila*. The majority of these isolates were toxic to gypsy moth and, more moderately, to lackey moth larvae. Variable degrees of virulence were instead observed on fly species. These results represent the first report of the insecticidal properties of novel and promising strains belonging to different bacterial species against a wide target range. Funding: this work was financially supported by Autonomous Region of Sardinia L.R. 7 Project 2012, cod. 60126 and by Banco di Sardegna Foundation, Project 2014, Prot. U158.2015/AI.131.MGB.

Poster. **BA-22**

Preparation and formulation optimization of a mosquitocidal sustained-release *Bacillus thuringiensis* with high UV-resistance

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Persistence of *Bacillus thuringiensis* products is an important factor in their application. In the present study, the preparation and formulation optimization were carried out to obtain a mosquitocidal sustained-release Bt with high UV-resistance. LLP29-M19 is a mutant from the mosquitocidal Bt LLP29 during ultraviolet mutagenesis, which is high UV-resistant and toxic against mosquitoes. Statistical methods were employed to analyze and optimize its sustained- release formulation. According to the single-factor experiments, the most suitable concentrations of sodium alginate, CaCl2 and hollow glass bead were 1.0%, 2.0% and 3.5%, respectively. Plackett-Burman design revealed that CaCl2, sodium alginate and hollow glass bead were the three key factors in the sustained-release formulation. The steepest ascent experiment was used to get the optimal region of the formulation composition. Then the optimal combined concentration and mutual effect of the three factors were optimized by response surface methodology (RSM). The result showed that the best formulation composition was sodium alginate 0.78%, CaCl2 4.52%, hollow glass bead 3.12%, bacterial powder 3.0%, melanin 0.015%, sodium benzoate 0.2%, mice feed 0.5%, immobilized time 4.5 h, at which the corrected sustained-release virulence rose up to 2391.67, which was 5.07-fold higher than basic formulation and just deviated 5.0% from the predicted value by RSM.

Poster. **BA-23 STU**

Resistance of different *Spodoptera frugiperda* populations to Bt-maize from the Bahia and Góias states correlates with low alkaline phosphatase expression

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Transgenic crops producing *Bacillus thuringiensis* (Bt) toxins used in agricultural pests control for insect have been successful, but their efficacy is reduced when pests evolve resistance. The *Bacillus thuringiensis* mode of action of 3d-Cry toxins involves sequential interactions with several insect midgut proteins that facilitate the formation of an oligomeric structure and induce its insertion into the membrane, forming a pore that kills midgut cells. Cry toxin binding to insect midgut epithelial receptors is an important determinant of specificity. Many putative Cry toxin receptors have since been reported, of which the best characterized are the aminopeptidase N receptors and alkaline phosphatases. The objective of this study was to further analyze the *S. frugiperda* population of the detection of ALP and APN activity of six Brazilian populations of *S. frugiperda* collected in the field, in the State of Bahia and Goias, Brazil. We analyzed and compared with the susceptible colony of *S. frugiperda*. This breeding was implemented in 1988 and the insects have never been exposed to Bt toxins. This study the detection of the midgut receptors of the larvae of *S. frugiperda* (BBMV) was analyzed by determining the specific activity of ALP and APN by SDS-PAGE gel using the substrate for detecting the activity, and also western-blot with ALP and APN antibodies of *Manduca sexta*. These data indicate that the levels of ALP receptors detection and the APN of some of the resistant populations showed low levels of ALP in all analysis, which is a low activity of this receptor when compared to the susceptible population. Demonstrating that the insect populations may be involved with ALP receptors, but not with APN, in these Brazilian populations.

Poster. **BA-24 STU**

Spent Juncao substrate can be converted into fermentable sugar with one-step method

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Juncao, which can be used as a culture medium, is favorable to cultivate edible or medical fungi. In order to solve the environmental and economic problems caused by spent Juncao substrate (SJS) which has cultivated various edible fungi, adopting three pretreatments to convert SJS into the reducing sugar and selecting the optimal one-step method, including dilute sulfuric acid (0.5–4% w/v, 50–121 ◦C, 1 h), sodium hydroxide solution

(0.5–2% w/v, 50–121 ◦C, 1 h), and hot water (50–121 ◦C, 1 h). The crystal structure, morphological, chemical composition of raw and pretreated SJS were appraised by X-ray diffraction (XRD), scanning electron microscope (SEM) and inferred from Fourier transform infrared spectroscopy (FTIR). They showed the highest yield of reducing sugar (243.8 g/kg SJS) and the strongest cellulose degradation of SJS compared to untreated SJS that was produced by 4% dilute sulfuric acid pretreatment at 121 ◦C. Therefore, the utilization of SJS not only provide a foundation for converting reducing sugar to cultivate Bacillus thuringiensis (Bt), but also solve the environmental and economic problems for Juncao industry.

Poster. **BA-25**

Structural analysis of mosquitocidal toxin sequences from a *Bacillus thuringiensis* native strain

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Insect-borne diseases, particularly those transmitted by mosquitoes, are among the leading causes of mortality and morbidity in humans. Therefore, the management of the insect populations is a critical step in the control of the diseases they transmit. The application of different management strategies of mosquito populations is essential for the control of these viral and parasitic diseases. In a previous work, we characterized an Argentinian *Bacillus thuringiensis* strain (FCC 41) that exhibited mosquitocidal activity against *Aedes aegypti*, *Culex pipiens*, *Culex apicinus* and *Ochlerotatus albifasciatus*, and harbored Cry24Ca and Cry50-like proteins in its parasporal body. In this work, we analyzed the proteins coding sequences present in FCC 41 strain and described the presence of *orf2* -like sequences downstream the *cry* genes, identified as *cry40-orf2* -like and *cry39-orf2*-like respectively. Structural analyses based on protein models were performed using bioinformatics tools and the hypothetical domain regions were identified, particularly the presence of exposed regions of domain II. We will clone and express *cry* genes and *orf-2*-like regions in heterologous systems in order to determine their toxicological activity and function in the crystallization and/or stability. These toxins could be used for the insect vector control programs of public health importance. Supported by ANPCyT (PICT No 2013-0431), CONICET (PIP 112 20110100963) and Universidad Nacional de Mar del Plata Project (15/E692 - EXA742/15).

Poster. **BA-26**

Study of Bacillus thuringiensis Cry toxin binding sites in the two important soya pests *Anticarsia gemmatalis* and *Chrysodeixis includens*

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Competition-binding studies performed with 125-Iodine labelled proteins demonstrated the presence of specific binding sites for the *Bacillus thuringiensis* (Bt) toxins Cry1Ac and Cry1Fa on the midgut brush border membrane vesicles (BBMV) of two important soybean pests: *Anticarsia gemmatalis* (velvetbean caterpillar) and *Chrysodeixis (=Pseudoplusia) includens* (soybean looper). Shared and unshared binding sites were detected for these Bt toxins in BBMV of larvae from both insect species. This information is important to take into account to ensure long term crop protection when designing pyramided transgenic plants involving more than one Bt protein. In an effort to explore the nature of the BBMV binding sites of Cry1Ac and Cry1Fa in *A. gemmatalis* and *C. includens,* different sugars and lectins were used to study their ability to inhibit the Cry1Ac and Cry1Fa specific binding to BBMV. Our results suggest that Cry1Ac and Cry1Fa have unique binding sites in the BBMV in addition to shared binding sites. Competition-binding studies further indicated that Cry1Ac or Cry1Fa binding sites were not shared with Cry1Ca or Cry2Aa in either soybean pest, similar to previous reports for other Lepidopteran insect species. This study contributes to the knowledge of Bt toxin binding in *A. gemmatalis* and *C. includens* and the cross-resistance potential for these Bt proteins as candidates for insect-resistant soybeans.

Poster. **BA-27**

Synergism of Cry1Ac and Cry1Ie toxins and its potential for resistance management

Kanglai He, Fan Jiang, Tiantao Zhang, Zhenying Wang;, IPPCAAS, Beijing, China

A number of insecticidal crystal proteins such as derived from *Bacillus thuringiensis* are toxic to lepidopteran pests such Asian corn borer, *Ostrinia furnacalis* (Guenée), the most destructive insect pest of corn in China and some part of Asia. In addition, the relative toxicities of these individual Cry toxins vary widely among species. However, the mixtures of functionally diverse toxins might be more effective than single toxins and might also delay evolution of resistance in target insects. Toxicities of Cry1Ac, Cry1Ie, and their mixtures were examined through diet incorporate bioassays. Synergistic effects of two toxins were tested using B.E. Tabashnik's methods (1992). The potential performance for resistance management was evaluated through protein bioassays and breeding stacked *cry1Ie* and *cry1Ac* corn plant tissue bioassays against laboratory selected resistance Asian corn borer larvae. The mixtures of Cry1Ie and Cry1Ac were more toxic (1.5- to 5-fold against susceptible, Cry1Ie-selected, and Cry1Ac-selected Asian corn borer larvae, respective) than expected on the basis of the potencies of its components. Tissues bioassays data indicated that hybrid with pyramiding two genes showed more effective insecticidal efficacy to Cry1Ac- and Cry1Ie-selected larvae, but hybrids expressing a individual toxin would be less effective to its correspondingly resistant larvae including those with cross resistance. The results suggested that there was a synergism between Cry1Ac and Cry1Ie toxins. Stacking these two genes has the potential for managing or delaying the evolution of resistance to Bt corn in Asian corn borer.

Poster. **BA-28**

Temperature restriction in *Photorhabdus luminescens*

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Temperature plays an important role in bacteria-host interaction and can be a determining factor for host switching. Here we investigate the reasons behind growth temperature restriction in the entomopathogenic bacterium *Photorhabdus*, which may be preventing some species from making the switch to a human host. *Photorhabdus* has a complex symbiotic and pathogenic life cycle. The genus consists of three species, only one of which, *P. asymbiotica* that can grow at 37 ◦C, is able to cause human disease. The other two species, *P. luminescens* and *P. temperata*, are largely

restricted to growth temperatures below 34 ◦C. We have observed that as opposed to *P. temperata*, which cannot replicate at 37 ◦C at all, *P. luminescens* TTO1 is able to undergo a few cell divisions before entering stationary phase at this temperature. Additionally, we have isolated clones of *P. luminescens* TTO1, that were able to fully grow at 37 ◦C on agar plates. Following whole genome sequencing of 29 such clones we identified a single gene, encoding a RecG-like helicase that contained single nucleotide polymorphisms in the majority of the clones. The gene appears to be part of an operon, which we have termed the Temperature Restricting Locus (TRL). TRL is absent from *P. asymbiotica* and its presence is not uniform in different strains of *P. luminescens*. We are currently trying to understand why *P. luminescens* TTO1 cannot sustain normal growth at 37 ◦C and the role of the TRL operon in this process. This may help us comprehend the evolutionary mechanisms that allow adaptation of pathogens to different hosts.

Poster. **BA-29 STU**

Use of *Caenorhabditis elegans* as model for selection of *Bacillus* spp. toxic strains to *Meloidogyne incognita* race 3

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The culture of cotton (*Gossypium hirsutum*) is affected by various diseases of major economic importance, and, one of them is the root-knot nematode (*Meloidogyne incognita* races 3). *Bacillus* are natural antagonists of several plant pathogens, but *in vitro* tests for selection of strains with potential for biocontrol of nematodes are compromised due to the difficulty to obtain high nematode populations. Given this, the present work had as objective to evaluate the potential of *Caenorhabditis elegans* as a model organism for selection of strains of *Bacillus* spp. biocontrol potential against *M. incognita*. To this end, a test *in vitro* was conducted with 11 strains of *B. thuringiensis* obtained from various soils and ecosystems to check the toxic activity in the nematode *C. elegans*. Later, these bacterial strains were tested against J2 of *M. ingonita*. The *C. elegans* test was mounted in sterile Petri dishes with 7.5 mL of *C. elegans* and 2.5 mL of bacterial suspension. After applications of treatments, the dishes were settled in incubator (21◦C) without light, for 72 h, and evaluations were made under optical microscopy. The experiment was settled in a completely randomized design with 15 treatments and 3 replications. For *M. incognita* tests, the nematodes were multiplied in tomato plants and then extracted. *M. incognita* eggs were extracted on Baerman funnel to obtain the J2 larvae. The assembly of *M. incognita in vitro* trials was identical to the ones for the *C. elegans*. The most efficient *Bacillus* strains *in vitro* were toxic to *C. elegans* and *M. incognita*. These bacterial strains significantly reduced the populations of both nematodes. These data suggest the possibility of using the *C. elegans* model as preliminary selection test of *Bacillus* against *M. incognita*.

Poster. **BA-30**

Vip3Aa laboratory selection and characterization of resistance in *Heliothis virescens* (Lepidoptera: Noctuidae)

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Laboratory selection with Vip3Aa of a field-derived population of *Heliothis virescens* produced > 2040-fold resistance in 12 generations of selection. The Vip-Sel resistant population showed little cross-resistance to Cry1Ab and no cross-resistance to Cry1Ac. Resistance was unstable after 15 generations without exposure to the toxin. F1 reciprocal crosses between Vip-Unsel and Vip-Sel indicated a strong paternal influence on the inheritance of resistance. Resistance ranged from almost completely recessive (mean h = 0.04 if the resistant parental was female) to incompletely dominant (mean h = 0.53 if the resistant parental was male). Results from bioassays on the offspring from backcrosses of the F1 progeny with Vip-Sel insects indicated that resistance was due to more than one locus. Brush border membrane vesicles from Vip-Unsel and Vip-Sel larvae showed similar binding of Vip3Aa, suggesting that binding alteration is not the basis of resistance.

POSTER SESSION — Wednesday, 10:30-13:30 – *Agnès Sorel*

Diseases of Beneficial Invertebrates division

Poster. **DB-1-STU**

A New Phylogeny and eDNA Insight into Paramyxids:
An Increasingly Important but Enigmatic Clade of Protistan Parasites of Marine Invertebrates

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Paramyxids (Rhizaria; Ascetosporea; Paramyxida) are parasites of marine molluscs, annelids, and crustaceans. The best known paramyxid is *Marteilia refringens*, which causes Aber disease (marteiliosis) of oysters, and also infects other bivalves. However, paramyxids are of more general and increasing interest, as they cause other commercially significant diseases, e.g. QX disease of Sydney rock oysters caused by *Marteilia sydneyi* and marteilioidosis of oysters and clams in Korea & Japan (*Marteilioides chungmuensis)*, and are being described as new taxa and emerging diseases on a regular basis (e.g. *Marteilia cochillia* in Spanish cockles; *M. octospora* in Spanish razor clams) as interest in the group grows. This study reviews the diversity, host affiliations and geographical ranges of all known paramyxids, and presents a comprehensive phylogeny of the order. Environmental DNA sequencing using paramyxid-specific primers shows that paramyxids are associated with a wider range of hosts and habitat types than previously known. Histology, electron microscopy and *in situ* hybridisation techniques link sequence data with species previously only known from morphological studies.

Poster. **DB-2**

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- Towards empirical identification of pattern recognition receptors (PRRs) from different hosts -Host-pathogens interaction relies on the recognition of several membrane-associated proteins from the pathogen by the host. The pathogen associated molecular patterns (PAMPs) are recognized by immune receptors to engage immune response pathways. These receptors must be carried at the surface of immune-cells in hemolymph or free in the plasma. In order to identify these proteins, interactomic studies are often performed. They generally consist in the extraction of proteins from the host and from the pathogen; then they are incubated together and the resulting proteomic profile is analyzed after differential centrifugation. In order to cope with this species-specific, long and hard to analyze approach, we propose an alternative approach: Realistic: freshly extracted cell-free hemolymph are directly put in contact for a short time (20') with the entire pathogens. Only outer membrane-bound proteins of the pathogen are exposed and recognized by native soluble proteins from hemolymph, as it would be within the host. Simple: the protocol is highly reproducible and straightforward, from extraction to 2D-gel profiling, and qualitative analysis only takes few hours. Universal: once the protocol has been developed for one pathogen, any invertebrate host can be tested.

Poster. **DB-3**
Cancelled

Poster. **DB-4**

Honey bee immunity: Its modulation by dietary supplements and probiotics

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Honey bees are important pollinators that are used by human for several thousand years. The immune system of honey bees can be divided to cellular and humoral branch that are complemented by highly developed social behaviour of bees. The activity of some immune factors is low in honey bees compared to other insects which is supposed to be caused just by social immunity. We tried to improve immunocompetence of honey bees by feeding them by selected plant extracts from *Echinacea* sp. and *Leuzea* sp., plant alkaloid sanguinarine and by administration of probiotic bacterial strains (*Lactobacillus apis*, *L. melliventris* and *Gilliamella apicola* isolated from gut of honey bees). To evaluate the health condition of treated honey bees we followed several physiological and immune parameters including protein concentration, antimicrobial activity, phenoloxidase activity and antioxidant capacity of hemolymph. We detected high variability in some of the followed parameters and confirmed lower antimicrobial activity of honey bee hemolymph compared to other insects. Furthermore, after treatment with tested compounds the honey bee larvae were collected and subjected to nematobacterial infection to confirm immunostimulatory effect of tested substances. We observed better survival rates upon probiotics and sanguinarine treatment of honey bees suggesting that they are suitable supplements for improvement of honey bee health. On the other hand, no significant effect on resistance was observed after feeding the plant extracts. Our research is supported by grant from the Ministry of Agriculture of Czech Republic (project no. QJ1210047) and Technology Agency of Czech Republic (project no. TA04020318).

Poster. **DB-5-STU**

Identification of *Serratia marcescens* infection in industrial rearing of *Tenebrio molitor*

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INRA- Micalis, Jouy en Josas, France

Industrial insect rearing goal is to produce large amount of high quality insects, in a short time, following safety, secure and sanitary conditions. Insect rearing diseases are caused by pathogens that compromise insect quality and production. Few things are known on those pathogens, on their diversity, their virulence, their prevalence and on ways to detect them. In this study, we focus on *Serratia marcescens* infectious signs detection in an industrial farming of *Tenebrio molitor* developed by Ynsect. Epizootics are well-known in insect farming and have already been detected in our rearing. *S. marcescens* is a red-pigmented gram-negative enterobacterium considered as a potential or facultative pathogen. It is not pathogenic when present in the gut but once in the hemocoel, it multiplies causing lethal septicaemia. Stressful biotic or abiotic events can weaken insect and facilitate *S. marcescens* infection. This study aims to identify factors that can facilitate *S. marcescens* infection and then to develop methods for an early detection of contaminated insects in mass production. Several series of challenging tests were set up on *T. molitor* larvae: (1) by direct subcuticular injection, (2) by *S. marcescens* contaminated feed ingestion (3) by co-infection with *S. marcescens* and virulent strain of *Bacillus thuringiensis* inoculated feed and finally (4) by stressors and *S. marcescens* contaminated feed ingestion combination. In this last assay, we test abiotic factors (light exposure, starvation, density, injuries, temperature, humidity, agitation) and combine them to study their impact on *Tenebrio* survival, feed consumption and growth performances. The results will allow us to develop molecular detection tools like PCR and qPCR.

Poster. **DB-6-STU**

Identification of the honeybee parasitic mite *Varroa destructor* resistance using discrimination concentrations of acaricides *in vitro*

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The honeybee parasitic mite *Varroa destructor* is the major driver of honeybee colony losses. Successful beekeeping depends on acaricide treatment of honeybee colonies against *V. destructor* to keep low population of parasitic mite. Synthetic acaricides are widely used from 80's, when *V. destructor* introduced and spread across all Europe. We propose to test varroa mites to distinguish sensitive and resistant populations to acaricides. We adapted plastic vial bioassay for identification of resistant populations of *V. destructor* in the Czech Republic. The sensitivity of three different Czech varroa mites populations to tau-fluvalinate, acrinathrin and amitraz were observed in vial bioassay. The acaricidal compounds were diluted in acetone and applied to the vials. The females of varroa mites from infested larvae and pupae were added into vials for 24h. The populations of mites differed in their sensitivity to three tested acaricidal compounds: pyrethroids acrinathrin (A) and tau-fluvalinate (B); and formamidine amitraz (C). Kyvalka population was resistant to A, B and C, while Postrizin were sensitive to all A *in vitro*. The intermediate situation was in Prelovice population, where the mites were sensitive to A and C, but not to B. When LC50 concentrations for A were compared, sensitive population (Postrizin) has 85, 91 and 31 times lower concentrations than resistant population (Kyvalka) for B, A and C, respectively. The suggested discrimination concentrations are following: 1, 0.3 and 0.2 μg/mL for B, A and C, respectively. The application of discrimination concentrations could help for early detection of varroa resistant populations. The study is supported by the project QJ1530148 of the Czech Ministry of Agriculture.

Poster. **DB-7-STU**

Occurrence of Gammaproteobacteria in honey bee gut infected by *Paenibacillus* larvae

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There is growing evidence supporting the importance of gut microbiota in relationship of common pathogens. However, little is known about the role of each specific strain and variance of the microbial composition in gut of infected bees. Gammaproteobacteria one of the eight typical phylotypes includes two species *Gilliamella apicola* and *Frischella perrara* frequently detected in gut of the honey bee. Thus the aim of our work was to understand if there is any possible influence of *Paenibacillus larvae* as causative agent of American foulbrood on the prevalence of *G. apicola* and *F. perrara* compared with other bacterial strains based on statistical comparison of Denaturing Gradient Gel Electrophoresis (DGGE) fingerprints. Total digestive tracts of infected and healthy foraging bees were removed. DGGE of 16S rRNA was conducted and profiles were compared using BioNumerics 6.6 software. We performed redundancy analysis where the first canonical axis clearly distinguished health bees in the left area from infected on the right, showing higher proportion of *Gilliamella* and *Frischella* strains on the left, compared to other undetermined strains on the right. Results indicate that Gammaproteobacteria was less abundant in honey bees infected with AFB than in non-infected, suggesting a negative interaction between these pathogens and these two Gammaproteobacteria strains. Potentially, the gut symbionts confer affect immune function, physiological enzyme activities or play a role in biofilm formation and constitutional microbiota establishment. Understanding of bacterial interactions would be useful in developing new probiotics. Our research is supported by grant from the Ministry of Agriculture of Czech Republic (project no. QJ1210047).

Poster. **DB-8**

Ontogeny of the immune system in harlequin ladybird, *Harmonia axyridis*

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The harlequin ladybird, *Harmonia axyridis*, is considered to be one of the world's most important invasive insects. It has been spread over substantial part of Europe and North America in last decades and its invasion is potentially detrimental for native ladybird species. The powerful immune system makes harlequin ladybird highly resistant to diseases that affect other ladybird species and is probably one of the main reasons of its successful invasiveness. In this study, we examined the basic immune parameters including concentration of haemocytes and antimicrobial activity in haemolymph during ontogenetic development (from the 2nd larval instar to the 32 days old adults). The concentration of haemocytes in haemolymph of *H. axyridis* does not change significantly during larval stages and is quite low until the prepupal stage (about 5500 haemocytes per 1 μl of haemolymph). After the metamorphosis the concentration of haemocytes rapidly increases during first eight days of adulthood and then it remains constant (around 30000 haemocytes per 1 μl of haemolymph). Similarly to the haemocyte level, the protein content in haemolymph was significantly influenced by the metamorphosis which caused the temporal decrease in protein concentration between prepupa and newly hatched adults. Subsequently, the protein concentration gradually increased within first 16 days of adulthood. The haemolymph of *H. axyridis* exhibited very high antimicrobial activity against *Escherichia coli* which increased from larval stages till adults. Surprisingly, we did not detect any significant differences in measured parameters between sexes of *H. axyridis* adults.

Poster. **DB-9-STU**

Pathogens of *Carcinus maenas* in their invasive range

Jamie Bojko[1], K. Fraser Clark[2,3], Kelly Bateman, Sarah Stewart-Clark, Rose Kerr[4], Ronny Van Aerle[4], Stuart Ross, David Bass[4], Alison Dunn[5], Paul Stebbing[6], Grant Stentiford

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European shore crabs (*Carcinus maenas)* are a globally invasive crustacean species, classed as one of the world's 100 'worst invaders'. Several invasive populations of *C. maenas* have been implicated in negative environmental and ecosystem outcomes including impacts on local biodiversity and as pests to aquaculture species (such as oysters and soft shell clams) on the Atlantic Coast of Canada. In attempts to control this invasive species, a licensed fishery was launched in two Canadian provinces. To date, the main market for these invasive crabs has been as a bait source for the lucrative ($1bn pa-1) lobster (*Homarus americanus*) fishery. *Carcinus maenas* has been shown to host 70 known pathogen taxa in its native and invasive ranges with frequent novel associations being recorded. Given a potential for the transmission of pathogens carried by *C. maenas* used as bait, to lobster, it is timely to consider the pathogen profile of this crab within its invasive range in Canada. Here, we followed the proposed invasion pathway of *C. maenas* into Eastern Canada via the Faroe Islands and used this pathway to screen for known and novel pathogens using histology, transmission electron microscopy and molecular diagnostic tools. During the study a novel microsporidian, amoebae and several viruses were discovered for the first time in crabs sampled from locations in Faroes and Canada. The screen also provided evidence for shared bacterial and acanthocephalan parasites between the crab and *H. americanus.* The study highlights potential for pathogen acquisition and loss by invasive hosts in new environments and provides evidence for the inclusion of pathogen risk assessments if invasive hosts are to be utilised as baits.

Poster. **DB-10-STU**

Ultrastructural analysis of antennal gland in American lobster experimentally infected with White Spot Syndrome Virus

Louise-Marie Roux[1,2,3], Philip Byrne[3,4], Fraser Clark[1,2], Glenda Wright[2], Dorota Wadowska[5], Spencer Greenwood[1,2]

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White Spot Syndrome Virus (WSSV) is one of the most virulent pathogens affecting shrimp aquaculture worldwide. The World Organization for Animal Health (Office International des Epizooties, 'OIE') considers all decapod crustaceans susceptible to WSSV. In shrimp, WSSV targets ectodermal and mesodermal tissue with cuticular epithelium, gill, and the epithelial lining of stomach being prominent sites of infection. Tissue tropism of WSSV in the American lobster (*Homarus americanus)* is poorly documented. Previous research found the American lobster produces a targeted immune response to experimentally created WSSV infection involving intramuscularly injected virus, at 20ºC. WSSV DNA or virions were present in many tissues, based on qPCR, histology or TEM. The present study utilized light and transmission electron microscopy to elucidate the general ultrastructure as well as to characterize changes associated with WSSV virions, in the antennal gland (AG) of American lobster experimentally infected with WSSV. The AG from WSSV-infected and control lobsters were examined 1 and 2-week post inoculation. The AG is comprised of two main regions, the coelomosac and labyrinth. The coelomosac contains elongated cells with extensive apical blebbing. The labyrinth is composed of tall columnar cells with microvilli. Among infected animals, the labyrinth cells contained hypertrophic nuclei with marginated host chromatin that revealed the presence of WSSV rod-shaped and enveloped virions. In marine decapods, the AG is involved in osmoregulation and excretion. This study provides insight into the role that the antennal gland may play as a preferred site of viral replication and excretion in decapods.

POSTER SESSION — Wednesday, 10:30-13:30 – ***Agnès Sorel***

Fungi division

Poster. **FU-1**

A new species of *Moelleriella* (Clavicipitaceae, Ascomycota) based on morphological and molecular data from China

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Based on morphological and molecular data, a new *M oelleriella* species, *M. sinensis* sp. nov. collected from Yunnan of southwestern China, is described and illustrated in this paper. *Moelleriella sinensis* is characterized by its yellowish-brown subglobose stromata containing more lipids and surrounded by hypothallus but without paraphysis in its irregular pycnidium. Phylogenetically, the new species belongs to a distinct clade sister to the *M. macrostroma/M. sloaneae* clade.

Poster. **FU-2**

A new xanthone derivative from a new isolate of the entomopathogenic fungus *Moelleriella* sp.

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This report describes the isolation of a new polyketide-dibenz[*b*,*e*]oxepin-11(6*H)*-one-1,10- dyhydroxy-3-methyl-7,8-dimethoxy-from a submerged culture of the entomopathogenic fungus *Moelleriella* sp. (isolate MDYS-27). The structure of the new compound is elucidated based on one- and

two-dimensional NMR spectroscopic and mass spectrometric data. The new compound generally has moderate antifungal, antibacterial, and antioxidant activity but has high activity against the fungus *Mycogone perniciosa*, i.e., its IC50 value against *M. perniciosa* was 1.3 μM. The new compound has the potential to become a new and effective fungicide.

Poster. **FU-3-STU**
Cancelled

Poster. **FU-4**

Characterization of an α-amylase from the honey bee chalk brood pathogen *Ascosphaera apis*

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The insect pathogenic fungus, *Ascosphaera apis*, is the causative agent of honey bee chalk brood disease. Amylases act to break down starch and, aside from their biotechnological application, are secreted by many plant pathogenic fungi to access host nutrients. Production of amylase by *A. apis* in submerged culture was optimized using the response surface method (RSM). Media composition was modeled using Box-Behnken design (BBD) at three levels of three variables. Experimentation demonstrated that the model developed successfully predicted amylase activity (R2 = 0.9528). Amylase activity was highest (45.28 ± 1.16 U/mL, mean ± SE) in media composed of 46 g/L maltose, 1.51 g/L CaCl2, at a pH of 6.6, in which amylase activity was 11-fold greater as compared to standard basal media. The enzyme was purified to homogeneity with a 2.5% yield and 14-fold purification. The purified enzyme had a molecular weight of 75 kDa, and was thermostable and active in a broad pH range (> 80% activity at pH range from 7-10), with optimal activity at 55°C and pH = 7.5. Kinetic analyses revealed a *K* m of 2.13 mg/ml and *V* max of 1.44 mg/min/ml using soluble starch as the substrate. Activity was significantly stimulated by Fe2+ and completely inhibited by Cu2+, Mn2+ and Ba2+ (10 mM), and significantly inhibited by ethanol and chloroform (10% v/v). The purified amylase essentially exhibited activity only on hydrolyzed soluble starch, producing mainly glucose and maltose, indicating that it is an endo-amylase (α-amylase). Amylase activity peaked at 99.38 U/ml fermented in a 3.7 L-bioreactor (2.15-fold > than what was observed in flask cultures). These data provide a strategy for optimizing production of enzymes from fungi and provide insight into the α-amylase of *A. apis*.

Poster. **FU-5**

Characterization of the pathogenicity of commercial or precommercial *Beauveria* sp. strains against the melon fly *Bactrocera cucurbitae*

Clara Rohrlich[1,2,3], Isabelle Merle[2], Magali Payet-Hoarau[2], Hugues Télismart[2], Samantha Besse[4], Samuel Nibouche[2], Laurent Costet†[2]
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The melon fly *Bactrocera cucurbitae* is a major pest of vegetable and fruit crops in Asia, in several African countries and Pacific islands.
The aim of this study was to evaluate the pathogenicity to *B. cucurbitae* of three strains of *Beauveria* used in commercial or precommercial formulations: Bb147 (Ostrinil^{QR}), I1081aa (*B. bassiana)* and Betel (*B. hoplocheli)*. Laboratory reared flies were dipped in a spore suspension at 10^6 spores/ml. Each insect was then placed in an individual housing box with food and water and the mortality was recorded daily for 30 days. Each strain was tested on males and females. Each test was replicated three times, each replication using a 30 fly cohort 8.5 to 11.5 days-old. Survival curves were modeled using the Kaplan-Meier model and pair-wised comparisons were carried out, using the log-rank test. The three strains were pathogenic to *B. cucurbitae.* The most pathogenic strains were the two *B. bassiana* with a lethal time 50 (LT50) of 7 and 6 days for I1081aa and 11 and 7 days for Bb147, respectively on males and females. The *B. hoplocheli* strain appeared significantly less pathogenic than the two *B. bassiana* strains, with LT50 of 19 and 18 days respectively on males and females. The strain I1081aa exhibits the best potential for biocontrol of *B cucurbitae*. Current work is considering this strain for the development of an Attract and Contaminate strategy. This work is supported by the French Ministry of Agriculture, Agri-Food and Forestry (MAAF), the French Ministry for Ecology, Sustainable Development and Energy (MEDDE) and received the ONEMA financial support under the Ecophyto PSPE2 project ”AttractMyFly” associating Cirad, Arysta LifeScience (Natural Plant Protection, Betel Reunion), AB7 Innovation and ARMEFLHOR

Poster. **FU-6**

Does *Agriotes obscurus* avoid the fungal entomopathogen, *Metarhizium brunneum*?

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Fungal entomopathogens can greatly reduce the fitness of their hosts, and it is therefore expected that susceptible insects will be selected to avoid exposure to pathogens. *Metarhizium brunneum* is a fungal pathogen that can infect *Agriotes obscurus* (Coleoptera: Elateridae), which in its larval form is a destructive agricultural pest. Assays conducted to determine the infective distance from mycosed cadavers demonstrated that beetles require direct contact with mycosed cadavers, or with soil that contacted mycosed cadavers. The ability of beetles to avoid direct contact with infective mycosed cadavers was examined using behavioural assays.

Poster. **FU-7**

Effect of temperature on germination, radial growth and spore production of different isolates of *Beauveria bassiana*

Medea Burjanadze, Mariam Arjevanidze, Giuli Tsereteli, Dali Gaganidze, Mikheil Gogebashvili
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For successful development as microbial control agents, entomopathogenic fungi have to be adapted to the environmental conditions. Temperature is an important environmental factor influence on the efficacy of entomopathogenic fungi. EPF. Definition of the optimal temperatures for growth rate and spore production is essential to the selection of fungal isolates well suited to the environment in which they will be used. For established thermotolerance of four local isolates of *Beauveria bassiana* Bb002, Bb027, Bb114, Bb(ED) from different geographical sites and ecological condition of Georgia (5-20000 m a.s.l.), were evaluated key physiological characteristics, as at three different temperatures 20,

25 and 30°C. In the experiment radial growth and spore production was assessed. At 20°C , minimum vegetative growth of 7.5 -11.4 mm was observed in Bb 002 and Bb 026 on 5th day, and maximum of 47.5-63.5mm on 15th day. Maximum values for radial growth were observed in case of Bb114 (66.5 mm), BbED (77.5mm) on 15 day. a maximum of $2x10^7$/ml spore output was recorded in BbED isolate. At 25 °C , highest radial growth of 90.5 and 96.2 mm were observed on 15th day in BbED and maximum of $1x10^9$/ml spore output was recorded. The percentage of conidia germination for all isolates was above 80 % in 30°C on 15th day and maximum of $5x10^8$/ml spore output was recorded in BbED isolate.

Poster. **FU-8-STU**

Effects of Metarhizium anisopliae (Hypocreales: Clavicipitaceae) on the food consumption and mortality of Mexican fruit fly, *Anastrepha ludens* (Diptera: Tephritidae)

Ricardo Toledo Hernández, Jorge Toledo Arreola, Daniel Sánchez Guillén
1 El Colegio de la Frontera Sur (ECOSUR),Tapachula, Chiapas, Mexico

Anastrepha ludens (Diptera: Thepritidae) is an important mango and citrus pest in México. We present a study on the effect *Metarhizium anisopliae* (Metsch.) Sorokin on food consumption and mortality of adult flies in laboratory conditions. The flies were treated with conidia using a hand-held spray bottle under laboratory conditions. We found that *M. anisopliae* was pathogenic to *A. ludens* (92.3% mortality). Also flies consumed less protein and sucrose after inoculation a sublethal dose of *M. anisopliae*. These results indicate the possible potential of the fungus *M. anisopliae* in biological control of *A. ludens*.

Poster. **FU-9**

Efficient production of *Aschersonia* placenta protoplasts for transformation using optimization algorithms

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The insect pathogenic fungus, *Aschersonia placenta* is a highly effective pathogen of whiteflies and scale insects. Few genetic tools, however, are currently available for this organism. Here we report on the conditions for the production of transformable *A. placenta* protoplasts using an optimized protocol based on the response surface method (RSM). Critical parameters for protoplast production were modelled by using a Box-Behnken design (BBD) involving three levels of three variables, and subsequently experimentally verified in its predictability of protoplast production (R2 = 0.9465). The optimized conditions resulted in the highest yield of protoplasts (4.41 ± 0.02 ×107 cells/ml of culture, mean ± SE) when fungal cells were treated with 26.1 mg/ml of lywallzyme for 4 h of digestion, and subsequently allowed to recover for 64.6 h in 0.7 M NaCl-Tris buffer. The latter used as an osmotic stabilizer. The yield of protoplasts was approximately 10-fold higher than that of the non-optimized conditions. Generated protoplasts were transformed with vector PbarGPE containing *bar* gene as the selection marker. Transformation efficiency was 300 colonies/mg DNA/107 protoplasts, and integration of the vector DNA was confirmed by PCR. The results show that rational design strategies (RSM and BBD methods) are useful to increase the production of fungal protoplasts for a variety of downstream applications.

Poster. **FU-10-STU**

Entomopathogenic fungi to control simultaneously both *Myzus persicae* (Green peach aphid) and plant diseases

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The green peach aphid, *Myzus persicae* Sulzer, is an economically important pest for greenhouse crops because they cause direct damage by feeding on plant nutrients and indirect damage as transmits many virus vectors. It has recently become a serious problem because of the continuous use of insecticide resulting in resistance among green peach aphid population. To overcome these problems, we screened the entopathogenic fungi against green peach aphid. Twenty isolates could be selected from 342 isolates of entopathogenic fungi. Recently, several species of entomopathogenic fungi have been shown to have multiple roles in nature as endophytes, they prevent plant pathogens from proliferating in the rhizosphere, and possibly even plant growth promoting agents. The antimicrobial activity of selected 20 isolates, therefore, were evaluated to plant pathogenic bacteria *Ralstonia solanacearum* and fungi *Botrytis cinerea* using dual culture technique on PDA media. Additionally, activities of these fungal culture filtrates were also tested using 96 well plate assay method to these pathogens. Various antimicrobial activities were observed against *R. solanacearum* or/and *B. cinerea*. Consequently, these entomopathogenic fungi would be used effectively for dual control agents against the green peach aphid and plant diseases.

Poster. **FU-11STU**

Evaluation of entomopathogenic fungi as the dual control agents against both *Tetranychus urticae* (Two-spotted spider mite) and plant pathogens

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The two-spotted spider mite, *Tetranychus urticae*, is one of the most important pests of fruit, vegetable and other plants in the world. Problems with this pest are becoming increasingly serious to plants. Chemical acaricides have been relied upon to control this mite for a long time. However, these are not always effective and their continuous use has resulted in resistance among two-spotted spider mite populations. To reduce or replace use of chemical acaricides, the entomopathogenic fungi having acaricidal activity were investigated. As results, 12 isolates could be selected from 342 isolates of entopathogenic fungi against two-spotted spider mite. Recently, several species of entomopathogenic fungi have been shown to have multiple roles in nature as endophytes, they prevent plant pathogens from proliferating in the rhizosphere, and possibly even plant growth promoting agents. The antimicrobial activity of selected 12 isolates, therefore, were tested to plant pathogenic bacteria *Ralstonia solanacearum* and fungi *Botrytis cinerea* using dual culture technique on PDA media. Additionally, activities of their fungal culture filtrates were also examined using 96 well plate assay method to these pathogens. Various antimicrobial activities were observed against *R. solanacearum* or/and *B. cinerea*.

Consequently, these entomopathogenic fungi would be used effectively for dual control agents against the two- spotted spider mite and plant diseases.

Poster. **FU-12**

Genetic diversity of *Metarhizium* spp. In grass, wheat, and forest habitats

Juerg Enkerli[1], Salome Schneider[2], Johanna Mayerhofer[1], Zhihong Huang[3], Ingvar Sundh[2], Stefan Vidal[4], Franco Widmer[1]
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Species of the fungal genus *Metarhizium* are important natural regulators of insect populations. They are globally distributed and can be isolated from a range of different habitats. Due to their ability to infect and kill insects, they represent great options for biological pest control. Based on recent multilocus phylogenetic analyses, the taxonomy of the genus *Metarhizium* has been thoroughly revised. For instance, the species complex *Metarhizium anisopliae* has been subdivided into nine species. Abundance and genetic diversity of these different *Metarhizium* spp., particularly in the light of the recent taxonomic changes, have remained unclear and knowledge on how they are affected by environmental factors or agricultural practices is limited. The goal of this study was to investigate abundance and population structure of the different *Metarhizium* spp., in three habitat types, i.e., wheat, permanent grassland and forest. Twenty soil cores were drawn along four parallel transects of 100 m each in each habitat and *Metarhizium* spp. were isolated using a selective medium (Strasser et al. 1996). A total of 74, 69 and 77 isolates were obtained from the wheat, permanent grassland and forest habitat, respectively. Genetic analyses using microsatellite markers allowed discrimination of 20 multilocus genotypes (MLG) of which 13 and 3 were affiliated to M. brunneum and M. robertsii using species-specific PCR. Three, 7 and 4 MLGs were identified exclusively in wheat, grassland and forest, indicating habitat specific population structures. Results will be compared to data obtained from corresponding samplings in Sweden and in Germany to investigate differences in Metarhizium spp. abundance and population structure across Europe.

Poster. **FU-13**

Genetic structure of *Beauveria bassiana* in different habitats of a holm oak tree

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The objective of the present study was to investigate the genetic structure of the entomopathogenic fungus *Beauveria bassiana* on a holm oak tree and its direct surroundings. Thirty-nine *Beauveria bassiana* isolates were collected from three different habitats, i.e., from the phylloplane (epiphytes and endophytes) from the soil and the leaves of weeds underneath the tree canopy. The genetic structure of the *Beauveria bassiana* population was studied using two different molecular tools: i) Elongation factor 1 alpha (EF-1α) sequence, and ii) Simple Sequence Repeat (SSR) marker analysis, using 14 SSR markers. The results revealed a high degree of phylomorphism among the 49 *B. bassiana* isolates, indicating considerable genetic variability in the population. Eight different EF-1α sequence types (STs) were detected, which all were identified as *B. bassiana sensu stricto* based on sequence alignments with *Beauveria* spp. reference sequences. The distribution of STs among the three habitats varied. On one hand, 3 STs (44 isolates) were isolated from soil (8 isolates), weeds (12 isolates) and the phylloplane (24 isolates) of the tree (epiphytes and endophytes). On the other hand, 3 STs (1 isolate each) were detected in the tree phylloplane and 1 ST (2 isolates) in the soil only. SSR analysis resulted in 32 multilocus genotypes (MLGs). The distribution of the MLGs corresponded to the ST distribution, i.e. 2 MLGs were detected in all the 3 habitats, 25 MLGs detected in the phylloplane and 5 MLGs in soil only. Results of the study suggested that *B. bassiana* genotypes may exhibit different ways and levels to interact with habitats of a single holm oak tree, some may inhabit multiple habitats and some may be restricted to single habitats only

Poster. **FU-14**
Cancelled

Poster. **FU-15**

Laboratory and field bioassays with *Beauveria bassiana* and *Metharizium anisopliae* against bark beetles

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Six isolates of *B. bassiana* (Bb) and one *M. anisopliae* (Ma) isolate were tested against the European spruce bark beetle, *Ips typographus,* adults in laboratory assays at a dosage of $1.5x10^6$ conidia. Mortality rates of *I. typographus* reached 100% at 4 days post treatment with 619Ma, 638Bb and 639Bb. The same isolates plus 562Bb were evaluated as the most virulent with LT50-values 2.616-2.901 days. Field bioassays were then conducted using isolates 562Bb, 638Bb and 619Ma in the Vitosha Mountain area of Bulgaria. Spruce logs were treated with conidial suspensions (106 conidia/cm^2). Three months later, bark was peeled from the logs and 1,126 beetles belonging to 10 Coleopteran species (Curculionidae and Cerambycidae) were collected, identified and analyzed for fungal pathogens. Analyses revealed natural occurrence of *B.bassiana*, *B.caledonica* and *Isaria farinosa.* Mortality rates of beetles collected from logs treated with 562Bb, 638Bb and 619Ma were 3.88%, 23.08% and 30.56%, respectively, and isolates 638Bb and 619Ma were significantly different from controls with $P = 0.001$ and isolate 562Bb from the control with $P = 0.05$. We showed the potential to inoculate bark beetles with entomopathogenic fungi by treating spruce tree logs.

Poster. **FU-16-STU**

Mealworm beetle - *Tenebrio Molitor* L as one of the best insect for isolation entomopathogenic fungi from soil

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The soil habitat is excellent habitats and reservoirs of diverse strains for insect pathogenic fungi which infect a range of invertebrate pest and have a cosmopolitan distribution, with potential for use in biocontrol. To detect insect pathogenic fungi in soil, various selective media have been used,

which approximated the density of fungal propagules in soil. The "Galleria bait method" first introduced by Zimmermann (1986), is as a sensitive method to detect a broad spectrum of insect pathogenic fungi in soil samples. The objectives of the present study was evaluation of insect bait method, using Mealworm - *Tenebrio molitor* for the isolation entomopathogenic fungi from soil. The studies, conducted during 2012-2015, shown that using *Tenebrio molitor* larvae as a bait insect is successful method for detection insects pathogenic fungi in soil. The comparing the wax moth-*Galleria mellonella* L. larvae with *T. molitor*, the last one is more active, moving into the soil, which could increase the chance of infection by entomopathogenic fungi. Additionaly, breeding *Tenebrio molitor* in laboratory condition is more easy, extremely simple and cheaper. The most abundance species isolated entomopathogenic fungi using *Tenebrio molitor* as bait insect were *Beaiveria bassiana* (48.5%), *Metarhizium sp.* (31.5%), Lecanicilum sp (2%), other Isaria sp (4%), Aspergillus flavus (3%) and other (11%).

Poster. **FU-17**

Moelleriella fujianensis sp. nov. (Clavicipitaceae, Ascomycota) from southeast China

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Moelleriella fujianensis, a fungal pathogen infecting whitefly nymphs (*Hemiptera)*, is described and illustrated as a new species from Wu Yi Mountain in Fujian province, China. This species is characterized by producing a yellowish-orange to orange spore mass around whitish to pale yellow pulvinate stroma. In surveys of entomopathogenic fungal diversity, only the anamorphic state was found in the natural reserve. Phylogenetic analyses of nuclear ribosomal large subunit rRNA gene, RNA polymerase b-subunit one and translation elongation factor 1-α coupled to morphological characterization support the placement of the isolate as a new species in a distinct lineage within *Moelleriella.*

Poster. **FU-18**

Molecular characterization of indigenous _Beauveria bassiana_ associated with coffee berry borers in Hawaii and assessments of their epizootic potential

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The coffee berry borer (CBB) is considered one of the most important insect pests of coffee worldwide. Females attack coffee berries, resulting in fallen berries or damage to the seed, which is the basis of the multibillion dollar coffee industry. As part of an area wide program to control the CBB in Hawaii, we are conducting a survey of indigenous *Beauveria spp.* associated with the beetle and assessing their prevalence in fields where a commercial strain of *Beauveria bassiana*, GHA, has been applied. We are also testing the virulence of representative indigenous haplotypes against CBB adults. Surveys of infected CBB from cultivated coffee farms and feral coffee at different altitudes in the South Kona district of Hawaii Island were conducted in 2014 and 2015. *Beauveria* isolates were characterized initially using colony morphology to differentiate GHA from indigenous conspecifics. This facilitated the rapid assessment of the prevalence of GHA in sprayed commercial farms. Molecular characterization of indigenous isolates using eight microsatellite markers and sequencing of the intergenic Bloc region revealed at least 10 haplotypes, representing five Bloc types of *B. bassiana* s. s. Molecular data also verified the use of colony morphology to initially identify GHA isolates. Preliminary prevalence data indicate that observed mycoses in coffee farms at the beginning of the new crop cycle are predominantly due to the indigenous strains, with GHA being reestablished following resumption of spray applications. Bioassays of the more prevalent indigenous *B. bassiana* haplotypes against laboratory-reared CBB also revealed virulence comparable to that of GHA.

Poster. **FU-19**

Natural occurrence of wireworms (Coleoptera: Elateridae) and entomopathogenic fungi in sunflower fields of Spain, and evaluation of their pathogenicity toward wireworms

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The wireworms *Agriotes* spp. (Coleoptera: Elateridae) are important worldwide soil-dwelling pests of a large variety of crops such as sunflower. Their control has been previously achieved using insecticides banned in Europe now, due to their undesired negative effects on the environment and human health. The absence of suitable insecticides for the control of wireworms requires the search of new eco-friendly management alternatives. Entomopathogenic fungi (EF) have shown their great potential to control soil-dwelling insects because the soil provides them shelter from the environmental constraints. Therefore, EF could be a good candidate for biological control of wireworms both as inundative field applications and as sunflower seed dressing. The current work was developed in Andalusia, the major sunflower growing area of Spain, and natural presence of EF, as well as geographical distribution and incidence of wireworms, was evaluated. Natural presence of EF was determined by using colony forming unit (CFU) methodology. Additionally, pathogenicity of EF was determined on *Galleria mellonella* larvae and the most virulent ones were tested against wireworms. Preliminary results showed a low incidence of wireworms in the prospected fields. On the contrary, 46% of the fields showed the presence of several species of the most important entomopathogenic genera (*Metarhizium, Beauveria, Purpureocillium, Bionectria* and *Paecilomyces)*, which showed significant differences in the mortality rate of *G. mellonella* larvae. The most virulent isolates tested on *G. mellonella* and wireworms belonged to *Beauveria* genera, and their results are very promising for subsequent experiments.

Poster. **FU-20**

Production of blastospores by Brazilian strains of four entomopathogenic fungi using submerged liquid culture fermentation

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Production by solid state fermentation of *Metarhizium rileyi, Hirsutella thompsonii*, *Colletotrichum nymphaea* and *Lecanicillium lecanii* is limited and this is one of the factors that have impaired their commercial use. Liquid fermentation is an alternative to produce blastospores of these fungi. The objective of this study was to investigate the blastospores production of *L. lecanii* (5 isolates), *H. thompsonii* (1), *C. nymphaea* (3) and *M. rileyi* (4).

The media used contain acid hydrolyzed casein as the nitrogen source and glucose as carbon source. Blastospore concentration was determined daily in 250 mL Erlenmeyer baffled flasks containing 50 mL of culture media. All culture experiments were run in triplicates and experiments were conducted at least three times on different dates. Culture of all isolates of these species produced concentrations higher than 1 x 109 blastospores mL^{-1} after two or three days. The isolates ESALQ4947 of *M. rileyi,* presented the fastest growth, reaching 3 x 109 blastospores mL^{-1} in the second day.

Poster. **FU-21**

Secreted lipase as a molecular marker for *Beauveria bassiana*

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Cultures of *Beauveria* isolated from different sources and locations in Russia in 2000-2014 were used for multilocus sequence typing. Fungal species were previously identified using sequences of intergenic locus B which is a standard marker for *Beauveria* (Rehner et al., 2011). In spite of sampling fungi in a broad longitudinal range from Baltic Sea to Pacific Ocean, only two species have been identified among over 100 cultures, being *Beauveria bassiana* (*ca* 30 % of cultures) and *Beauveria pseudobassiana* (*ca* 70 % of cultures). The most frequent molecular haplotype of *B. bassiana* identical to Genbank entry under accession number HQ880696 (from type *B. bassiana* isolate ARSEF 1811) was present across the majority of geographical locations and ecological habitats studied (Lednev et al., 2014). For better understanding of intraspecific polymorphism, we designed primers for a dozen of putatively polymorphic genes encoding protein toxins, hydrolytic and oxidative enzymes for three isolates belonging to this Bloc haplotype from South-Western Russia, Western Siberia and Far East, respectively. Among the analyzed loci, secreted lipase (*slip)* was chosen for further studies due to stable PCR signal in all samples, clear sequencing result and appropriate level of nucleotide changes in the examined gene region of *ca* 450 bp. Sequencing *slip* gene in a broad range of *B. bassiana* cultures allowed clear differentiation of isolates originating from different locations and insect hosts which could not be differentiated using Bloc genotyping approach. We presume that *slip* can be a useful tool for further studies of *Beauveria* phylogenetics and strain genotyping. The research is supported by Russian Foundation for Basic Research (# 15-34-20567-mol a ved).

Poster. **FU-22**

Self-Defense: Insect pupal cells with antibiotic properties

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Soil-dwelling insects have developed various mechanisms to defend against pathogen infection. For instance, many insects spend long periods in pupal cells where they may be exposed to disease causing agents. We hypothesized that the cell may possess antimicrobial properties. The pecan weevil, *Curculio caryae*, spends two to three years in the soil inside an earthen cell. In a laboratory study, we tested the hypothesis using the fungus *Beauveria bassiana* as a model. *B. bassiana* is a common endemic pathogen of *C. caryae*. We compared the number of colony-forming-units on selective media when *B. bassiana* was exposed to autoclaved soil, non-autoclaved soil, or soil from a *C. caryae* pupal cell. Soil from *C. caryae* cells was suppressive to *B. bassiana*. Subsequently, to determine the breadth of the phenomenon, we tested whether pupal cells formed by other insects also exhibit antibiosis. Our results indicated that the pupal cells of the Diaprepes root weevil, *Diaprepes abbreviatus*, the deodar weevil *Pissodes nemorensis,* and plum curculio, *Conotrachelus nenuphar* all showed antibiosis by inhibiting *B. bassiana*. The findings expand our knowledge of host-pathogen relationships. Additional research is needed to determine the basis for the suppressive effects observed.

Poster. **FU-23**

The complete genome of *Metarhizium rileyi*, a key fungal pathogen of Lepidoptera

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The fungus *Metarhizium rileyi* (= *Nomuraea rileyi)* is a key regulatory agent of lepidopteran populations. In soybean and cotton agroecosystems, it causes major epizootics decimating important lepidopteran pests such as velvetbean caterpillar, soybean loopers, green cloverworm, cotton leafworm and others. Despite its recent phylogenetic transfer to *Metarhizium*, *M. rileyi* displays unusually high specificity by infecting only lepidopteran species. The complete genome of *M. rileyi* was assembled *de novo* using short-read Illumina data from paired-end and mate- pair libraries. A total of 311 scaffolds were constructed (> 1 kb, N50 = 800 kb), with a total length of 31,007,635 bp. An *ab initio* annotation, using a set of 2,159 gene stuctures of *Metarhizium robertsii* ARSEF 23 to train the model, predicted a total of 10,880 genes coding for proteins in this genome. Orthologous genes were detected in *M. robertsii* and *M. acridum*:
4,806 orthologs in both species, 349 orthologs only in *M. robertsii*, and 301 orthologs only in *M. acridum*. In-paralogs were not counted. Divergent genes (4,534) with no shared orthologs were compared to annotation data for known functions and were categorized according to an enrichment analysis of function-related aspects. A remarkable and more numerous category of genes appears to be polyketide synthases, possibly involved in the secondary metabolism required for virulence to insect hosts. This study provides the genome sequence and annotation of *M. rileyi*. Comparative studies of its genome among different isolates and species could provide new insights on differential pathogenicity and improved understandings of its relationships to phylogenetically related species.

Poster. **FU-24**

Tradeoffs of immune system function with longevtiy as mediated by diet

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Despite natural selection for improved immune function, much variation remains in this phenotype, suggesting the existence of tradeoffs with other fitness characters. We study the evolutionary and physiological tradeoffs between immune system function and longevity, and the influence of

dietary environment on these tradeoffs. Our experimental system includes 28 populations of *Drosophila melanogaster* that have been experimentally evolved for differences in longevity, immune defense, and metabolites and that have sequenced genomes. To test immune function we examine resistance to the fungal entomopathogen *Beauveria bassiana*. Long-lived populations are more resistant to infection, but populations that are selected for increased resistance to fungal infection are shorter-lived than their controls. Populations that are selected for increased triglyceride levels do not have improved resistance, but dietary supplement that increases triglyceride levels does improve resistance. Overall, the relationships among body content, longevity, and immune defense are complex and our ongoing experiments are untangling these interactions.

Poster. **FU-25-STU**

Update of knowledge about *Leptolegnia chapmanii* as an agent of biological control of mosquito Aedes aegypti.

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Leptolegnia chapmanii (Seymour) Straminipila, Peronosporomycetes is a facultative pathogen of mosquito larvae. Isolates of this microorganism are restricted to North America and at Southern hemisphere in Argentine. The native isolates from Argentine CEP 010 and CEP 433 are currently included at fungal entomopathogenic collection in Centro de Estudios Parasitologicos y de Vectores (CEPAVE). Hosts range, its safety against non-target species and some optimal environmental conditions for its infection and development on mosquito larvae under laboratory conditions were previously determined. *Aedes aegypti* (Linnaeus) is the main vector of diseases such as yellow fever, dengue, Chikungunya fever, Zika, among others. Two fundamental aspects of the knowledge of *L. chapmanii* were focused. First one was some ecological aspects such as parasitism, persistence and dispersion in environments where naturally take place larvae of *Ae. aegypti*. With a single pathogen application in containers the fungus persisted about 6 weeks reducing mosquito population with differences in mortality rates between locations. Zoospores iability was also affected for the exposure to UV. *Leptolegnia chapmanii* was compatible with different concentrations of Neem oil and Diflubenzuron being able to produce viable zoospores in presence of these products with larvicidal effect. For its massive production an economical solid and liquid culture medium based on sunflower seed extract was compared with two traditional culture media used in lab. On solid culture medium, *L. chapmanii* grew in all cases producing posteriorly a higher quantity of zoospores from sunflower medium and showed a good growth patron.

Poster. **FU-26**

Virulence of selected entomopathogenic fungi against the olive fruit fly and their potential for biocontrol

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The olive fruit fly, *Bactrocera oleae* (Diptera: Tephritidae), is the most serious pest of cultivated olives worldwide. Its recent invasion into North America, specifically California, has initiated renewed interest in management strategies for this pest. Research into classical biological control has been a major focal point for controlling this fly. However, the use of entomopathogenic fungi (EPF), which infect, invade, and eventually kill their host, has remained largely unexplored for this pest. One of the commonest methods used in biological control is to select agents from areas that match closely the climate of the region infested in order to increase the chances that agents discovered will be climatically adapted if they are introduced. As Mediterranean climate is found in California, explorations for EPF have been conducted in the Mediterranean Basin, including Greece and France. Therefore, five strains of EPF were isolated from soil in olive orchards in Greece and Crete in 2002 and one strain was isolated from infested olives collected in South of France in 2015. All the strains were identified as either *Beauveria sp.* or *Metarhizium* sp. Using pupae collected in infested olives, we evaluated the virulence of the isolates under laboratory conditions by inoculating pupae directly or by inoculating the soil. Emergence rate was monitored daily, and dead pupae were removed, surface sterilized and placed in sterile plates to be inspected. Identification of the EPF were confirmed based on sequencing of the ITS region. Depending on the virulence we will measure during the next summer, subsequent studies will be conducted to evaluate specificity in quarantine and efficacy in the field, in the country of origin.

POSTER SESSION — Wednesday, 10:30-13:30 – ***Agnès Sorel***

Micobial Control division

Poster. **MC-1-STU**
Cancelled

Poster. **MC-2**

Aphicidal potential and virulence of *Lecanicillium* fungi from Argentina

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Mitosporic fungi of the genus *Lecanicillium* (Ascomycota; Hypocreales) are of particular interest as biological control agents for phloem-sucking plant pests including aphids. Bioprospection for these fungi in Argentina has given rise to a set of single-spore derived *Lecanicillium* strains isolated from a wide range of original hosts. For species-level characterization of *Lecanicillium* isolates, five genetic markers comprising one mitochondrial (NMS) and two nuclear (ITS, IGS) ribosomal RNA operon together with one mitochondrial (*nad1)* and one nuclear (*ef1 a)* protein-encoding sequences, has been employed. The aggregated information from these markers indicates that fungal isolates from Argentina mainly, but not exclusively belong to the *Lecanicillium* core species. Moreover, *Lecanicillium* strains has been investigated for fungicide sensitivity. Between strains differences in susceptibilities have been found to be important and not necessarily in line with systematics, making careful determination of sensitivity to agriculturally used fungicides an important criterion of biocontrol agent selection. However, the fungicidal polyketide compound soraphen has been found of outstanding activity against a wide variety of isolates from all species investigated. On the basis of these results, some strains have been selected for virulence bioassays against *Brevicoryne brassicae*. For each replicate 15 apterous adults were used. The applied dose was 1.107 conidia/ml and the application method was spray. After treated, aphids were placed on each seedling and mortality was controlled during the 10 days after application. The strains that caused the highest and the lower mortality were 155 (66.6%) and 182 (31%), respectively.

Poster. **MC-3**

Biofilm fermentation for the production of insect pathogenic fungi

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Currently, integrated pest management that aims to reduce the use of synthetic insecticides by considering all appropriate alternative methods, is promoted. In that context, insect pathogenic fungi could be developed as biopesticides in two ways: spores but also metabolites that are recognized as virulence factors. Targeting adequate fermentation method is an important requirement to obtain fungal products (i.e. spores, enzymes and metabolites) of biotechnological interest. The 'Microbial Processes and Interactions' laboratory has developed an innovative fermentation technology (referred to as 'biofilm fermentation') involving the growth of fungal biomass on inert supports that are immersed in a liquid nutrient medium. Confining fungal biomass on immersed inert structures provide a hybrid production system aiming to keep a solid-state physiology for sporulation and secretion of metabolites while controlling fermentation parameters. This research project proposes to transfer this technology to culture insect pathogenic fungi, such as *Metarhizium anisopliae*, for the production of spores and insecticidal metabolites. Development stages include the qualitative and quantitative characterization of its impact on insecticidal products as well as scale-up to industrial level.

Poster. **MC-4**

Biological control of *Tuta absoluta* (Meyrick) (Lep: Gelechiidae) by the use of entomopathogenic fungi

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Devastating insects constitute one of strains for cultivate tomato. Among these insects, tomato leafminer (*T. absoluta)* has been recently introduced in Algeria (2008), it constitute a challenge for both agricultures and scientists. Firstly, this insect is introduced without their natural enemies which may reduce their damage. Secondly, this species has developed insecticide resistance to many insecticides. To contribute to establish a control strategy for *T. absoluta,* we have made an inventory for their enthomopathogenic fungi. Two fungi were identified among others taken from adults and pupae. These fungi are *Aspergillus flavus* and *Fusarium* sp. A study was conducted in laboratory of plant pathology to recognize the The virulence and the efficiency of these antagonists. Two concentrations (104 and 106 spores/mL) of *Aspergillus flavus* and *Fusarium* sp. were tested on *T. absoluta* larvae (2nd & 3rd instar) to study the effect of these agents on larval mortality. All the treatments resulted in significantly higher mortality than control. The highest percentage of dead larvae (100 %) was recorded with the two concentrations at day 6. Key words: *Tuta absoluta* ; enthomopathogenic fungi; *Aspergillus flavus* ; *Fusarium* sp.; control strategy.

Poster. **MC-5**

BioZec - Development of a biological tick control agent based on an innovative attract-and-kill strategy

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Ticks are vectors for a multitude of diseases, e.g. Lyme disease and tick-borne encephalitis. In Germany eight to ten million people suffer from tick bites every year, most bites being caused by the species *Ixodes ricinus*. At present, there is no effective individual control measure against ticks available. Driven by the need for a sustainable reduction of the use of chemical pesticides, the overall aim of this project is the development of a novel biological control agent against ticks based on an innovative attract-and-kill strategy. The basis of the control agent is the attractive effect of CO2 combined with tick-specific attractants and aggregation pheromones, slowly released by a specifically tailored biopolymer capsule. The capsule system will be combined with a kill component, primarily an entomopathogenic fungus, which is able to infect and kill ticks as they come into contact with the capsules. First experiments dealt with the formulation of baker's yeast in Ca-alginate capsules to achieve a high dose controlled release of CO2. On-going investigations are focused on the selection and cultivation of virulent fungi strains from the genus *Metarhizium*, which have been in part isolated from indigenous dead ticks in Germany, testing of novel attractant and aggregation candidate substances, selected encapsulation techniques as well as drying and rehydration of these beads.

Poster. **MC-6-STU**

Detection of natural antagonists in *Drosophila suzukii* – a chance for biological control of the invasive insect pest?

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Drosophila suzukii Matsumura (Spotted wing drosophila, SWD) is endemic in East China and Japan but has been introduced to Europe and the US about one decade ago. In Europe, it has emerged to one of the most harmful invasive pests to commercially grown fruit while it prefers ripe fruits ready for harvesting. SWD has caught the attention for developing biological control mechanisms and systems. We are investigating the possible usage of microbial antagonists for biological control. Therefore, we examine the natural prevalence of parasites and pathogens like fungi, bacteria, viruses, microsporidia and other protista in drosophilids and test them on their antagonistic potential. Screening of early field populations from different locations in Germany, enabled through intensive monitoring and field sampling, did not yet lead to find natural antagonists, contrasting to findings from Japanese SWD samples. Since *D. suzukii* was introduced to the Western hemisphere the invader rapidly adapted to the new environment disadvantageously to the ecosystem because population regulating antagonists like symbionts, pathogens and predators are missing and if existing they lack to adapt to the new species. On the contrary, such antagonists can be found more frequently in the pest's country of origin. Microsporidia were already found in wild populations of *D. suzukii* and are currently used to re-infect laboratory SWD populations. In addition, microscopic and molecular investigations are conducted for determination and characterization. However, detection of antagonists from Europe

seems to be promising viewed by the aspect of weak host-pathogen adaptation. On that account, we also test the potential of non-species- specific pathogens.

Poster. **MC-7**

Can Endophytic Beauveria or Metarhizium Control the Wheat Stem Sawfly?

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Abstract not communicated

Poster. **MC-8-STU**

Development of nanoencapsulated, particle-based bait prepared with bioactive and biocompatible entomopathogenic agents for the control of leaf-cutting ants (*Atta* and *Acromyrmex* sp.) and its cultivated fungi (*Leucoagaricus gongylophorus*) as an eco-friendly alternative for sustainable agriculture

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Leaf-cutting ants (LCA) are one of the main defoliators of Central and South America, foraging on nearly 15% of the annual production of plant biomass to cultivate a fungus garden for food. Control strategies to manage this pest have been difficult due to their eusocial behaviour and ability to avoid foraging hazardous particles. We hypothesize that it is possible to obtain an efficient control of leafcutters by designing a bait supplemented with pathogenic microorganisms isolated fom their nest. Our goal is to develop a bait with nano–encapsulated entomopathogenic fungal spores, *Bacillus thuringiensis* (*Bt)* toxins and *Escovopsis* sp. spores for collapsing the garden in order to cause a global, sinergistic negative effect over colony health. Ingestion assays of 15 Bt strains isolated from ant colonies allowed us to identify three strains that caused 45–54% mortality, with median lethal concentrations of 9.97×10e1–5.86×10e7 µg/mL and 5.5 days as a median lethal time (LT50). In addition, six strains of *Metarhizium*, *Beauveria*, *Paecilomyces* and *Aspergillus* were recognized as infectious agents by virulence assays, causing 33–81% mortality, with sublethal doses of 4.28×10e1–1×10e5 spore/mL and LT50 of 5.5 days. We are currently performing palatability assays *in vitro* and on the field to determine the optimal bait composition, in order to attract LCA and mask the presence of the aforementioned pathogens. Current and future research include producing polymeric capsules, performing coexistence bioassays with different pathogen combinations to optimize their synergistic effect, and conducting ultrastructural analysis with electron microscopy to verify the toxicity of the treatment on the fungus garden and the brush border membrane of ants digestive tracts.

Poster. **MC-9**

Identification of new *Bacillus thuringiensis* (Berliner) isolates as biological control agents against *Ostrinia nubilalis* (Húbner) larvae

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The European maize borer *Ostrinia nubilalis* (Lepidoptera: Pyralidae) is one of the most widely studied agricultural pests in the world. This insect feeds on many different species of cultivated plants, particularly maize, and is is widespread in Europe, North America and North Africa. It has been recently reported as an important problem in greenhouse-grown vegetable crops in southern Spain. *Bacillus thuringiensis* (Bt) produces a wide range of proteins with insecticidal activity against different insect pests. Commercial Bt products serovar kurstaki (DiPel®) and aizawai (Xentari®) are currently being applied to control *O. nubilalis*. However, these products are based on strains that produce crystals comprising of a limited number of proteins (Cry1A, Cry1C, Cry1D and Cry2A). The continual use of these products can lead to development of resistance in insect populations, as observed for *Plutella xylostella* in the open field (Ferré et al., 1991, PNAS 88:5119–5123) as well as *Trichoplusia ni* in greenhouses (Janmaat and Myers, 2003, Proc. R. Soc. Lond. B Biol. Sci. 270:2263–2270). The aim of this study was to identify Bt strains that produce very active Cry proteins which can help to delay or avoid the development of resistance in *O. nubilalis* populations. The molecular and biological characteristics of the selected Bt proteins will be discussed.

Poster. **MC-10**

Impacts of entomopathogenic fungi on biology and behaviour of the invasive Brown Marmorated Stink Bug (Hemiptera, Pentatomidae)

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Halyomorpha halys Stal (Hemiptera, Pentatomidae) is an invasive stink bug coming from Eastern Asia. Besides causing important yields losses in orchards, crop and vegetable fields, it overwinters inside houses as agglomerates of dozens. In Europe, this Brown Marmorated Stink Bug (BMSB) was accidentally introduced in Switzerland in 2007 and has been later observed in surrounding countries (France, Italy, Germany). Based on the current US situation, we can reasonably fear that BMSB will have colonized most of Europe countries in less than a decade. If we already discern a few fungus strains that are efficient against BMSB, very little is known about their actual impact on the insect itself. So we investigated the influence of a fungal infection on insect development parameters and behaviour. Olfactometry was settled to investigate the dispersion and aggregation trends, while electropenetography was used to assess the feeding behaviour. Our results will be discussed to present changes according to healthy/fungus-infected status, in relation to potential biological control for that pest.

Poster. **MC-11**

Interaction of commercial products based on *Bacillus thuringiensis* and *Cotesia flavipes* (Hymenoptera: Braconidae) to the control of *Diatraea saccharalis* (Lepidoptera: Crambidae)

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The study was conducted in order to verify *Diatraea saccharalis* (Lepidoptera: Crambidae) larvae mortality and indirect effects on the natural enemy *Cotesia flavipes* (Hymenoptera: Braconidae) due to the use associated with *Bacillus thuringiensis* (Bt). Therefore, we used Bt-based products available in the market: Agree^{R}, BacControl^{R} Dipel^{R} and Btt090^{R} applied through artificial diet for sugarcane borer larvae. The mortality of *D. saccharalis* larvae was evaluated as well as the adverse effects on parasitoids after 7 and 14 days of exposure to the products. After 7 days, Agree^{R}, Dipel^{R} BacControl^{R} were the ones that caused higher mortality, respectively 63.3%, 52.5% and 30,3%, and Btt090^{R} showed results similar to control (4.2% and 5.8%). After 14 days, Dipel^{R} (100.0%), Agree^{R} (100.0%) and BacControl^{R} (55.0%) caused greater larval mortality *D. saccharalis*, respectively 100.0%, 100.0% and 55.0%, and Btt090^{R} caused just 37.5% of larvae mortality. Regarding the parasitoid, egg-pupal period, pupa period, sex ratio, number of adults, pupal viability, wing length and width were evaluated, and adult longevity. Among the biological parameters evaluated just the parasitoid adult longevity was affected by Btt090^{R} (1.1 day) and BacControl^{R} (1.2 day), being in the control 1.6 days. The mortality of *D. saccharalis* larvae exposed to tested biopesticides is related to the time of exposure. Btt090^{R} and BacControl^{R} caused indirect effects by reducing the longevity of *C. flavipes* after exposure to Bt ingested through artificial diet. greater larval mortality *D. saccharalis*, respectively 100.0%, 100.0% and 55.0%, and Btt090QR caused just 37.5% of larvae mortality. Regarding the parasitoid, egg-pupal period, pupa period, sex ratio, number of adults, pupal viability, wing length and width were evaluated, and adult longevity. Among the biological parameters evaluated just the parasitoid adult longevity was affected by Btt090^{R} (1.1 day) and BacControl^{R} (1.2 day), being in the control 1.6 days. The mortality *of D. saccharalis* larvae exposed to tested biopesticides is related to the time of exposure. Btt090^{R} and BacControl^{R} caused indirect effects by reducing the longevity of *C. flavipes* after exposure to Bt ingested through artificial diet.

Poster. **MC-12**

Isolation and characterization of *Bacillus thuringiensis* strain from *Podisus nigrispinus* (Hemiptera: Pentatomidae)

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Podisus nigrispinus is a predator of lepidopteran and its natural occurrence was observed in organic brassicas crops in Brazil preying on *Plutella xylostella* larvae, being an important natural control of this pest *Bacillus thuringiensis*. The aim of this research was to isolate and characterize *B. thuringiensis* strain from *P. nigrispinus* nymphs. For that it was used about 50 nymphs who consumed *P. xylostella* larvae that had previously ingested HD1 strain of *B. thuringiensis*. The guts were removed from predators and macerated in sterile saline solution (sodium chloride 0.9% solution) The macerate was transferred to flat glass tubes and subjected to hot water (heating to 80◦C and five minutes in ice, to eliminate vegetative cells). Samplesof each respective treatment were then diluted 10 and 100 fold in saline solution, an aliquot of 100 μl of dilution was distributed in Petri dishes containing culture medium. Characterization of *B. thuringiensis* strain based on it morphological, physiological, and biochemical parameters. PCR analysis was performed, using specific primers for *cry1* gene, that encoding against *P. xylostella* larvae. HD1 strain of *B. thuringiensis* was isolated from *P. nigrispinus* nymphs. Thus, we proved that the HD1 strain actually went to the third trophic level, but without causing negative effects to the predator. The results indicate that the association of HD1 strain with *P. nigrispinus* is feasible for the Integrated Pest Management of *P. xylostella*.

Poster. **MC-13**

Isolation and identification of a *Serratia marcescens* protease with toxic activity against larvae of *Phyllophaga blanchardi* (Coleoptera: Scarabaeidae)

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The bacteria *Serratia marcescens* (Enterobacteriaceae) is a soil inhabitant very often associated with infection to insects. Several *S. marcescens* strains were isolated from the hemocoel of diseased *Phyllophaga* spp (Coleoptera: Scarabaeidae) larvae collected from soils in Mexico. Oral and injection bioassays using healthy *Phyllophaga blanchardi* larvae fed with the *S. marcescens* isolate 81 (Sm81) showed that the strain caused anti-feeding effect and mortality. Similar effects were observed when cell-free broths from *S. marcescens* 81 were used for bioassays. The disease symptoms were abolished after the culture broths were boiled, suggesting the involvement of proteins in the toxic activity. An insecticidal protein of 50.2 kDa was purified to homogeinity by Q-Sepharose anion-exchange column from the Sm81 cell-free broth. The 50.2 kDa protein presented protease activity analyzed by skim milk agar plate and *in gel* zymography. The protease was proven insecticidal towards *P. blanchardi* larvae by injection bioassay. Analysis of the insecticidal protein by Tandem- mass spectrometry (LC-MS/MS) and blast homology search showed similarities to a Serralysin-like protein from *S. marcescens* spp. Serralysins are Zinc-metalloproteases, which are common in pathogenic bacteria, and are often associated with virulence. This insecticidal protein could be useful in agricultural biotechnology.

Poster. **MC-14-STU**

Molecular identification and biological activity of African isolates of PhopGV on *Tuta absoluta* larvae

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The Gelechiidae *Tuta absoluta* (tomato leaf miner) is a Lepidopteran pest, which causes serious damage in tomato crops and occasionally in potato in sub-tropical and tropical regions. Enthomopathogenic baculoviruses are considered as specific and potent biological control agents of insect pests. Phthorimaea operculella granulovirus (PhopGV) has been reported to be infectious to larvae of *T. absoluta*. Five PhopGV isolates from Tunisia, Yemen and Kenya (Tu1.11, Tu2.11, Tns16, Ym14, Ken13) have been tested for their biological efficacy against of *T. absoluta* and the potato tuber moth, *Phthorimaea operculella*. First bioassay results showed that *T. absoluta* larvae were sensitive to PhopGV isolates with different LC50 values. The five PhopGV isolates were tested for their genetic diversity in regard to *egt* (ecdestroid UDP transferase) gene sequence as a marker;

both Tunisian PhopGV isolates Tu1.11 and Tu2.11 were genetically polymorphic compared to the reference Tunisian isolate PhopGV-1346. Additional mutations appeared to be accumulated by Tu2.11 compared to Tu1.11. The isolates Ken13 and Ym14 seem to be also polymorphic compared to reference PhopGV-1346. Complete genome sequencing of the isolates is going on and will be reported. This research is supported by the Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) (project 12.1433.7-001.00)

Poster. **MC-15**

The use of auto-contamination-dissemination strategy for emerald ash borer (A*grilus planipennis*) population management and development of a molecular tool for tracking the released native *Beauveria bassiana* (Bb) isolate

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Since 2002, the exotic invasive wood boring beetle, emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) *(EAB)* has been spreading throughout eastern North America. To slow the spread of this insect, entomopathogenic fungi can play a significant role. We have been investigating the potential use of an auto-contamination-dissemination strategy to introduce a native isolate INRS-CFL-A of the fungal pathogen, *Beauveria bassiana*, into populations of EAB. We evaluate the use of fluorescent powders as a cost-effective method of tracking the dispersal of deployed INRS-CFL-A propagules in the EAB population and habitat. Additionally, typing and tracking of INRS-CFL-A dispersal in EAB populations in the vicinity of the released site was achieved using conventional mycological techniques (culturing), and by PCR using a novel strain specific molecular marker developed based on the Bloc intergenic gene of INRS-CFL-A. Three-year results will be presented.

Poster. **MC-16**

Using an Antarctic fungus as a wintertime biopesticide

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Scientists at the British Antarctic Survey and CABI in collaboration with AlphaBio Control are investigating whether cold-adapted fungi from the Antarctic can be used to kill overwintering insect pests in the UK. This project, supported by NERC Innovation, is testing an Antarctic fungus against black vine weevils (*Otiorhynchus sulcatus)* and large pine weevils (*Hylobius abietis)*, as well as investigating the ecology of the fungus *in vitro* in UK agricultural soils and, its impact on earthworms. The team identified a strong candidate zygomycete fungus from a genus not previously considered to be pathogenic to insects. During low temperature transport from the Antarctic to the UK the fungus was observed to overgrow and kill waxmoth larvae, it was also found to grow relatively quickly and reproduce at 10◦C. Acute toxicity tests of the present study considered filter paper testing and/or artificial soil substrate testing: significant mortality rates (> 80%) were achieved for black vine weevils in the soil substrate tests. There was no earthworm mortality in either soil or filter paper tests with the exception of the highest dose in the latter. Tests on large pine weevils are scheduled for later this year.

Poster. **MC-17-STU**

Virulence of vegetative insecticidal proteins Vip3Aa60 and Vip3Ad5 of *Bacillus thuringiensis* against *Spodoptera exigua* (Lepidoptera).

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Vegetative insecticidal proteins (Vips) secreted by *Bacillus thuringiensis* (*Bt)* strains during the vegetative growth stage, were seemed as the second generation of *Bt* insecticides. A number of *Bt* isolates were indicated in screening tests. Two novel *vip3A* genes with 2370 bp and 2361 bp in length were classified as *vip3Aa60* (NCBI accession No.: KR340473) and *vip3Ad5* (NCBI accession No.: KR263164), respectively. There were two mutations in both *vip* genes which led to two amino acids modification. Homology modeling results indicated that the two amino acids mutations of Vip3Ad5 (Met3 and Ala6) were both located in the loop, while the Met3 of Vip3Aa60 was in the loop and the Glu337 of Vip3Aa60 was in the *α*-helix. After expression of these vip genes, the proteins were purified and characterized. The toxicity of both toxins was estimated against lepidopteran pests of *Spodoptera exigua*. The results were interesting that Vip3Aa60 had high toxicity against *S.exigua* as the LC50 value was 28.9 ng/cm2 but Vip3Ad5 toxin displayed no toxicity to *S.exigua* yet. The insecticidal mechanism of Vip3Aa60 and Vip3Ad5 was further study from the proteolytic processing, the core peptidase stability and the mode of interaction with their receptors.

POSTER SESSION — Wednesday, 10:30-13:30 – ***Agnès Sorel***

Microsporidia division

Poster. **MI-1**

Experimental infection of *Loxostege sticticalis* (Lepidoptera: Pyraloidea) with microsporidia

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Progeny was obtained from field-collected *Loxostege sticticalis* moths reared under lab conditions. Second instar larvae were used for alimentary infections. Spores of *Tubulinosema* cf *loxostegi* isolated from *L. sticticalis* was highly infective to this host (up to 80 %) even after one year of storage of infected cadavers at ambient temperature. *Nosema pyrausta* from *Ostrinia nubilalis* were also highly infective to *L. sticticalis*, but presumed admixture of pathogenic microbiota caused early host mortality making impossible normal propagion of this microsporidium. Spores of *Nosema ceranae* and *Nosema* cf *granulosis* isolated from alive moths or fresh cadavers of *L. sticticalis* were caused infections in this host at lower degrees

(up to 20 %). *N. ceranae* spores isolated from midguts of alive honey bees were not infective to *L. sticticalis*. The research is supported by RSCF grant # 16-14-00005.

Poster. **MI-2**

Horizontal transmission of the microsporidium *Nosema adaliae*, from the two-spotted lady beetle, *Adalia bipunctata*, to the green lacewing, *Chrysoperla carnea*

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The green lacewing, *Chrysoperla carnea* Stephens, and the two-spotted lady beetle, *Adalia bipunctata* L., are natural enemies that are commonly used in biological control in North America. They are used in greenhouses and agriculture through augmentative release, and are mass-produced in commercial insectaries in Europe. Both have been found to host different species of microsporidia; however, *Nosema adaliae* has been described from *A. bipunctata* and has a chronic effect on its host. Due to coexistence of these two natural enemies in agricultural systems, there is potential for the horizontal transmission of *N. adaliae* from *A. bipunctata* to *C. carnea* when they are used simultaneously for biological pest control. The objective of this study is to determine whether or not *N. adaliae* is successfully transmitted through the consumption of *N. adaliae*-infected *A. bipunctata* eggs, and to quantify pathogen effects on *C. carnea* larval development. A combination of uninfected and *N. adaliae*-infected eggs was fed to *C. carnea* larvae, and development was observed over 30 days. Test larvae were examined upon death or after the 30 day for microsporidian spores by light microscopy. Pathogen transmission was low, suggesting that *C. carnea* is a poor host of *N. adaliae*. Even though most test larvae remained uninfected, larval development was delayed significantly for test larvae fed 2 or 3 microsporidian-infected eggs.

Poster. **MI-3-STU**

The anti-Nosema active substances from entomopathogenic fungal cultures

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The population of managed honey bees has been dramatically declining the recent past in worldwide. The one of most common disease of bees is nosemosis which is caused by microsporidia in the genus *Nosema*. *Nosema apis* and *N. ceranae* have been described as honeybee pathogens. These microsporidia are highly evolved fungi with an obligate intracellular parasitic lifestyle. The disease causes significant detriment to honey production and results in economic losses. In our knowledge, Fumagillin is the only antibiotic approved for control of nosemosis in honey bees, however this antibiotic may have unintended effects on the honey bee host, ultimately contributing to increased prevalence and pathogenicity of *Nosema*. To solve this limit, we screened anti-*Nosema* active substances from numerous entomopathogenic fungal culture filtrates and evaluated their efficacy to control *Nosema* disease in the honey bees. Entomopathogenic fungal culture filtrates were prepared from 342 fungal isolates and anti-*Nosema* active substances were screened using *in vitro* polar tube germination assay. Entomopathogenic fungal culture filtrates showing high active inhibition against *N. ceranae* were used to further quantitative analysis and *in vivo* honeybee bioassay. These fungal metabolites may be employed as antibiotic agents and a good feature to be used in the development of new biocontrol agents of nosemosis.

Poster. **MI-4-STU**

The effects of RNAi to microsporidian parasites *Nosema ceranae* in the honeybee

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Honey bees are one of the important insects in the world as pollinators of numerous agricultural crops. Honey bees have faced many diseases which threaten bee colony including a serious population decline phenomenon called Colony Collapse Disorder (CCD). *Nosema ceranae* is a pathogen cause nosemosis, also called Nosema, which is the one of most common disease of bees. According to the genome sequencing for *N. ceranae*, it has been identified that the presence of machinery for RNA silencing and RNA interference technology (RNAi) has successfully silenced the ADP/ATP transporter gene in this species under laboratory conditions. Microsporidia *N. ceranae* that are obligated intracellular parasites depend on their host for energetic and metabolic needs. Here we selected the several genes from mitosome of *N. ceranae* to develop RNAi for the control of Nosema. Especially, TOM40, FNR1, FNR2, and Nar1 were chosen because these show less homologous with those of the honey bee, *Apis mellifera*, and then each RNAi was synthesized. After infection of *N. ceranae*, the honey bees were treated with RNAi either by using only one or combining two or more. The infection rate and specific gene silencing in Nosema were analyzed. Effects of the chitosan-based nanoparticles in RNAi treatment were also investigated.

Poster. **MI-5**

The Proboscis Extension Response as a behavioral tool for assessing the vectorial competence along the life cycle of *R. prolixus* (Hemiptera: Reduviidae)

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American trypanosomiasis ("Chagas disease") is caused by infection with the protozoan parasite *Trypanosoma cruzi*. This zoonosis is a major public health problem in Latin America: 6 to 7 million people infected, potentially 100 million at risk. The infection is transmitted mainly by blood-sucking triatomine bugs, and among them, *Rhodnius prolixus*, the main vector. These bugs ingest the parasites when they feed on an infected vertebrate host that they will deposit later on a new host with their faeces during or shortly after their next blood meal. The vectorial capacity is therefore influenced by different parameters linked to the feeding behavior, such as the amount of blood ingested by meal or the defaecation delay. Our study examined whether the epidemiologic risk associated to *R. prolixus* could vary across the stages of the life cycle (5 larval instars before the adult). We investigated one trait of host- seeking: the response to the temperature of a potential host, the only necessary and sufficient cue to trigger the Proboscis Extension Response (PER) that precedes the bite. We assumed that if the temperature selected by bugs changes with the

developmental stage, this could reveal a shift in the host type impacting on the epidemiological risk for humans. The results showed that PER rates reach a peak around 30-35°C and decreased for a warmer temperature. This pattern of thermal preference was maintained all along the life cycle, adults included. However, males exhibited a decrease in the amount of responses whatever the temperature. These results associated with those measuring different parameters directly related to the transmission of *T. cruzi,* suggest a weaker vectorial competence in males *R. prolixus*, as compared to nymphs and females.

POSTER SESSION — Wednesday, 10:30-13:30 – ***Agnès Sorel***

Nematode division

Poster. **NE-1-STU**

An entomopathogenic nematode extends its niche by associating with different symbionts

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Bacterial symbionts are increasingly recognised as mediators of ecologically important traits of their animal hosts, with acquisition of new traits possible by uptake of novel symbionts. The entomopathogenic nematode *Heterorhabditis downesi* associates with two bacterial symbionts, *Photorhabdus temperata* subsp. *temperata* and *P. temperata* subsp. *cinerea*. At one intensively studied coastal dune site, *P. t. cinerea* is consistently more frequently isolated than *P. t. temperata* in *H. downesi* recovered from under the bare sand/*Ammophila arrenaria* of the front dunes where harsh conditions including drought prevail, but not in the more permissive closed dune grassland further from the sea. No differences were detected in ITS sequence between nematode lines carrying either of the two symbionts. The two symbionts could be readily swapped between lines, and both were carried in equal numbers within infective juveniles. In laboratory experiments, we tested whether the symbionts differentially affected nematode survival in insect cadavers that were allowed to dry. We assessed numbers of nematode infective juveniles emerging from insects that had been infected with *H. downesi* carrying either symbiont and then allowed to desiccate for up to 62 days. In moist conditions, cadavers produced similar numbers of nematodes, irrespective of the symbiont present, while under desiccating conditions, *P. t. cinerea* cadavers yielded more nematode progeny than *P. t. temperata* cadavers. Moreover, cadavers harbouring *P. t. cinerea* reduce the rate of drying of cadavers relative to those harbouring *P. t. temperata*. Our experiments support the hypothesis that *H. downesi* can extend its niche into harsher conditions by associating with *P. t. cinerea*.

Poster. **NE-2**

Biological control of large pine weevil with entomopathogenic nematodes: on the way to large scale application

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Large Pine weevil, *Hylobius abietis* (Coleoptera: Curculionoidea), is a significant pest of conifer forests throughout the Northern Europe. Weevils develop on stumps of clear-felled forests and they feed on the young seedlings upon emergence, causing high rates of mortality and malformations. The common way to control *H. abietis* is the use of chemical insecticides. Over the last years many researches focused on the biological control of *H. abietis* using entomopathogenic nematodes. In this study we report results on field trials conducted using *Steinernema carpocapsae* (Rhabditida: Steinernematidae) and *Heterorhabditis downesi* (Rhabditida: Heterorhabditidae) over the last two years in Ireland. We particularly examined how soil types and method of application affect nematode effectiveness in suppressing *H. abietis* population. Treatment efficiency was measured as both parasitism rates and emergence of adult weevils in traps. The next step in the development of a useful biological control *H. abietis* are large scale trials using spraying machine and tree damage assessment.

Poster. **NE-3**

Concurrent transcriptional profiling of *Dirofilaria immitis* and its wolbachia endosymbiont throughout the nematode life cycle reveals coordinated gene expression

Jeremy Foster, Ashley Luck, Silvia Libro, Barton Slatko; New England Biolabs (NEB), Ipswich MA, United States

Dirofilaria immitis (canine heartworm) is a filarial nematode related to human filarial parasitic nematodes. The *D. immitis* (*Di)* genome, along with the genome of its obligate endosymbiont, *Wolbachia* (*wDi)*, was recently completed. To help unravel the complex relationship of the nematode and e symbiont, we transcriptionally profiled *Di* and *wDi* throughout the ne matode life cycle. Over 215 million single-end 50 bp reads were generated from total RNA from five *Di* life cycle stages. Using hierarchical clustering, nearly 70% of all *Di* genes display stage-specific transcriptional patterns. Comparison of adult male (AM) and adult female (AF) samples reveals that over 1400 *D. immitis* genes display sex-biased transcriptional patterns. Among all five life cycle stages examined, a significant portion of *Di* genes are L4-associated (4375 transcripts), whereas only 58 transcripts show L3-biased expression. Pairwise comparison of the L3 and L4 transition stages reveals 1450 significantly differentially expressed genes (560 L3 upregulated, 890 L4 upregulated. A large proportion of both *D. immitis* and *wDi* genes display microfilarial-biased transcriptional patterns. While significantly fewer reads mapped to *wDi* genes than to *Di* genes in each life cycle stage, 653 *wDi* genes were deemed differentially expressed ($q < 0.01$) between at least two of the five life cycle stages. Synthesis of the critical metabolite, heme appears to be synchronized with the production of heme-binding proteins in a stage-specific manner. Comparative analysis to human filarial nematodes provides further information on the evolutionary biology of these parasites, while also highlighting opportunities for further drug targeting initiatives.

Poster. **NE-4**
Cancelled

Poster. **NE-5-STU**

FIM Track: a novel method for tracking of Drosophila larval behavior in response to entomopathogenic nematodes

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Drosophila melanogaster is one of the most widespread model organisms. It is used in various types of research fields involving genetics, immunology, developmental studies and others. There are also many research groups who focus on behavioural patterns of adults flies or their larvae. For this field a new method was developed called FIM Track (FTIR-based Imaging Method). It is based on frustrated total internal reflection (FTIR) and allows us to observe groups of *Drosophila* larvae, which are crawling at the surface of a translucent agar gel. Thanks to newly developed software, we can track larval movement and measure parameters such as velocity, area of larvae, accumulated distance, etc. These parameters are commonly used for evaluation of *Drosophila* responses to various stimulus (heat, light, food, parasite, etc.). We used FIM Track to study behaviour of third instar *Drosophila* larvae responding to entomopathogenic nematodes. We observed some differences in larval behaviour when parasites were present. Larvae tried to avoid the contact with nematodes by moving faster. They also tried to get rid of parasites from the cuticle by bending more frequently, twisting and rolling. Thanks to FIM Track we were able to observe the behaviour of *Drosophila* larvae with great resolution in real time enabling us to have a very close look at the interaction between larvae as hosts and their nematodal parasites. Our research is supported by grant from the Ministry of Agriculture of Czech Republic (project no. QJ1610248).

Poster. **NE-6**

Heme Acquisition in the Human Parasitic Filarial Nematode, *Brugia malayi*

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Human filarial nematode infections responsible for lymphatic filariasis affect nearly 150 million people. As heme auxotrophs, nematodes lack a heme biosynthetic pathway and must acquire heme from exogenous sources. Given the indispensable role of heme, this auxotrophy in nematodes may be exploited to develop drugs that interfere with heme uptake or utilization in parasites. To further understand the biology of heme metabolism in parasitic nematodes, we have undertaken the first study of heme transport in *Brugia. malayi*. We show the *B. malayi* orthologue of the *C. elegans* gene *Ce* HRG-1, *Bm* HRG-1, localizes both to the endocytic compartments and cell membrane when expressed in yeast cells. This dual localization bypasses the need of the *C. elegans* HRG-4 orthologue. Functional assays in yeast, heme analogue and RNAi experiments show that *Bm* HRG-1 is a functional heme transporter. Transcriptomics reveals that *Bm* HRG-1, *Bm* HRG-2 and *Bm* MRP-5 (*C. elegans* orthologues) are heme responsive. Other HRGs in *B. malayi* (*Bm* HRG-3, *Bm* HRG-4, *Bm* HRG-5 and *Bm* HRG-6) remain unidentified. While the precise mechanisms of heme homeostasis in a nematode with the ability to acquire heme from its environment as well as perhaps its *Wolbachia* endosymbiont remains unknown, the filarial nematode *B. malayi* is capable of transporting exogenous heme.

Poster. **NE-7**

Historical review of entomopathogenic nematode research in Korea

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Entomopathogenic nematode (EPN) research was introduced in Korea at 1988 by Dr. Choo in Gyeongsang National University. He studied from Dr. Kaya in UC Davis, USA. Eighty three EPN papers have been published in different scientific journals from 1988 to present. Among them, 70 papers were conducted by Dr. Choo (87.9% of all published manuscripts). Most of the EPN research of Dr. Choo included identification and control of pests, distribution, culture and physiology of nematode. Major research part was efficacy experiment against insect pests for using biocontrol agents (51.8%). Target pests were thirty seven insects and 1 plant parasictic nematode. Seven species (*Heterorhabditis bacteriophora*, *H. megidis*, *Steinernema carpocapsae*, *S. glaseri*, *S. kraussei*, *S. longicaudum* and *S. monticolum)* in 2 families of EPN were isolated and dominent species is *S. monticolum* in Korea. Two EPNs (*S. carpocapsae* and *H. bacteriophora)* were commercially produced in Korea. EPN market size enlarged from 2000 to 2010 however, decreased after then. Only small group of people are involved in EPN research in Korea. Recently EPN research has been forcused on application technology.

Poster. **NE-8-STU**

***In vivo* efficacy of *Heterorhabditis bacteriophora* on *Cephalcia tannourinensis*, pest of the Cedar natural forests of Lebanon**

Martine Rehayem[†1], Elise Noujeim[†2], Bernard Duvic[†3], Jean-Claude Ogier[†3], Olivier Thaler[†3]
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Cephalcia tannourinensis (Chevin) is a hymenopteran sawfly attacking the Cedars of Lebanon, *Cedrus libani*, putting in danger the national emblem of the country since 1990s. A biological control program using a commercial strain of *Heterorhabditis bacteriophora*, one of the commonly used entomopathogenic nematodes (EPNs) was considered due to its high efficacy in previous *in vitro* experiments on *C. tannourinensis* (Noujeim *et al.*, 2014). This program seeks to maintain this pest under its threshold level without affecting the ecosystem of these protected areas. *In vivo* experiments were conducted on *C. tannourinensis* larvae and the greater wax moth *Galleria mellonella* in jars introduced in the soil of the forest under natural conditions. Insects' mortality is assessed in order to determine the efficacy of the *in vivo* experiments while the emergence rate of the EPNs shows their establishment by reproducing and finishing their cycle in the insect. EPNs' persistence in the forest was determined 14 days after the application through the use of *G. mellonella* baiting technique to compare the efficacy and establishment of the EPNs *in vivo* to the *in vitro* experiments showing if the EPNs are capable of infesting, reproducing and emerging from *C. tannourinensis* larvae under natural conditions. The non persistence of the EPNs will explain the results of a previous prospection in this ecosystem where the non-detection of this parasite proves that it is not indigenous to the area of study (Noujeim *et al.*, 2011).

Poster. **NE-9**

Molecular Diagnostics of Human Nematode and Protozoan Gastrointestinal Parasites in Rural Argentina, with Impact on Intestinal Microbiota

Rojelio Mejia, Ashish Damania, Barton Slatko; New England Biolabs (NEB), Ipswich, United States

Over 2 billion people are infected with parasites that disrupt intestinal bacterial flora affecting nutrition. We used quantitative real-time PCR (qPCR) and NextGen sequencing analysis from microbiota enriched patient stool samples from rural Argentina for microbiota and 8 common GI parasites including the nematode helminths, *Ascaris lumbricoides* (Al), *Ancylostoma duodenale* (Ad), *Necator americanus* (Na), *Strongyloides stercoralis* (Ss), *Trichuris trichiura* (Tt) and protozoa *Cryptosporidium parvum* (Cp), *Entamoeba histolytica* (Eh) and *Giardia lamblia* (Gl). qPCR identified Al (56.6%) and 37.4% for two hookworm species (Na and Ad) with Na as the predominate hookworm, while Ad DNA was detected in higher concentrations (0.61 versus 119.6 fg/μL, $p < 0.0001$. For Gl and Ss, 63.6% and 21.2% were positive, respectively. qPCR was also able to detect polyparasitism of 2 or more parasites in 59% of patients. For GI samples, microbiome analysis data shows decrease in biodiversity in the parasite infected group compared to those non-infected, with a shift away from Firmicutes toward increased Bacteroides, with the degree of shift related to intensity of infection. Abundant bacteria within the Bacteroides were due to increased Prevotella, while the non-infected group had decreased Ruminococcus. Clustering between the groups was examined using PCoA ordination and Shannon alpha diversity. This first use of multi-parallel qPCR and NextGen microbiota sequencing shows an association of GI parasite infections and decreased microbiome biodiversity in symptomatic patients. The results will enable refinement of treatment options on a public health scale, leading to better health outcomes in endemic settings.

Poster. **NE-10**

Optimizing the efficacy of entomopathogenic nematodes for the control of annual bluegrass weevil, *Listronotus maculicollis*, larvae

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The annual bluegrass weevil (ABW), *Listronotus maculicollis*, is a major pest of short-mown golf course turf in the northeastern United States and eastern Canada and has become particularly problematic due to the development of resistance to commonly used insecticides. In previous studies we had found that entomopathogenic nematodes (EPN) can provide acceptable control (50-80%) of ABW larvae in golf course fairways. The most consistent control was provided by the species *Steinernema carpocapsae*, but *S. feltiae* and *H. bacteriophora* often gave the same level of control. In greenhouse and field studies we found that combinations of the neonicotinoid insecticide imidacloprid and the all three EPN species provided additive control. These combinations are very feasible since, when applied at the typical time for ABW larvae control, the imidacloprid component will also provide control of a complex of white grub species, another important turfgrass pest. We found that splitting the EPN applications in two equal half rates applied 5-7 days apart further improved the efficacy of *S. carpocapsae* alone and its combinations with imidacloprid, in the latter treatment providing up to 96% control. Similar results were obtained with insecticide-susceptible and –resistant ABW populations.

Poster. **NE-11-STU**

Pheromone mediated attraction and maturation in *Steinernema* adults

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Recent studies have shown that nematodes from a variety of taxa synthesise and communicate vital information using blends of small glycolipid molecules known as ascarosides. Biological activity of this novel class of pheromones unique to nematodes is dependent on which molecules are present and also their relative proportions within the blend. Entomopathogenic nematodes (EPN) of the dioecious genus *Steinernema*, are gaining favour as model organisms for studying traits relative to parasitism, sexual reproduction and for their commercial value as biological pest control agents. We show that female *Steinernema* emit chemical signals which both attract and initiate sexual development in conspecific males only, presenting considerations as to how species specificity is achieved chemically. As multiple species of EPN are capable of occupying a single insect host in unison, it is important to clarify how they may receive each other's chemical messages, particularly where non-native species may be artificially introduced as biocontrol agents. Furthermore, increased understanding of how chemical signals mediate vital reproductive and survival behaviours within the insect host will contribute to improving mass production and field efficiency of EPN as a viable alternative to traditional, and in many cases environmentally damaging, chemical pesticides.

Poster. **NE-12**

RNAi-mediated gene silencing of candidate drug targets in the filarial nematode *Brugia malayi*

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The filarial nematode *Brugia malayi* is one of the causative agents of lymphatic filariasis (elephantiasis), a neglected tropical disease that affects 120 million people in endemic tropical areas. Current treatments such as albendazole and ivermectin rely on mass drug administration (MDA) programs - but they predominantly target larval stages, necessitating a treatment course of up to 10–15 years. Due to this and other limitations of MDA, extensive effort has been devoted to research for alternative treatments. The increasing availability of genomic data allows to identify genes that are essential to the survival or reproduction of the parasite and can thus represent new targets for antifilarial drugs. Here we focused on chitin synthase, an enzyme that catalyzes the synthesis of chitin, a component of the microfilarial sheath and of the pharyngeal cuticle. In order to investigate whether chitin synthase is required for the nematode's viability and reproduction, we performed a RNA interference (RNAi)-mediated knock down of chitin synthase by exposing live adult worms to gene-specific siRNA oligos for three days. Worms exposed to a scrambled RNA sequence were used as a negative control to determine off-target effects. Every 12 hours worms were examined by microscopy for morphological alterations, microfilarial release by adult females, adult wormmotility and the MTT viability assay. Silencing efficiency was assessed by qPCR analysis of target transcript levels at each exposure time. The effects of siRNA treatment and negative control were compared to untreated worms.

Poster. **NE-13**

Selective DNA Enrichment, High Quality Library Construction and Quantitation For Robust NextGeneration Sequencing

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Next Generation Sequencing (NGS) technology has rapidly matured over the past few years. One goal continues to be advancement of the upstream processes required to produce maximum high quality and accurate sequence data with reduced DNA/RNA input. Recent Improvements in sample prep workflow include the development of the NEBNex^{R} Ultra II Library Prep kit with requirements as low as 500 pg DNA, the NEBNex^{R} Library Quant Kit, a simple and robust method for quantitation of Illumina libraries and the NEBNex^{R} MicroBIome DNA Enrichment Kit designed to eliminate or capture microbial or symbiont DNA from complex samples. Using a streamlined library preparation protocol which reduces time required to produce high quality DNA libraries and a concomitant reduction of the possibility of protocol errors, the NEBNext Ultra II method significantly improves library yields and reduces sequence bias from a variety of input templates, including G+C rich, FFPE and ”enriched” samples. Preparation times are as low as 15 minutes hands-on time and 3.2 hrs total workflow. The advantages offered by the NEBNext Library Quant Kit ensure optimal cluster density and consistency for a broad range of library types and sizes, by providing a cost-effective qPCR library quantitation workflow to optimize both sequencing yield and throughput. Based upon selective methylated (CpG) DNA capture, the NEBNext Microbiome DNA Enrichment Kit enables the separation of microbial DNA from a microbiome sample, which often contains a mixture of microbial and host DNA. An example of this combination approach is our analysis of divergent intestinal microbial communities among parasite-infected Argentinian children.

Poster. **NE-14-STU**

Soil as a Habitation of Biological Control Agents for Pest Management

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This study provides a search and isolation of entomopathogenic agents from different soils and their action to pest insects in the natural environment. The soil samples were collected from different ecosystems in Georgia: vineyards, fruit orchards (Kartli Kakheti), citrus orchards and windbreaks (Guria, Samegrelo, and Adjara), Forests (Tskhneti, Kiketi), potato growing plots and unpopulated areas in High Mountains (Mtskheta-Mtianeti, Svaneti) etc. The soil samples were processed for isolation of entomopathogenic nematodes (EPN) and entomopathogenic fungi (EPF) by using the accepted methods in insect pathology (Stock, & Goodrich-Blair, 2008a, Humber, 1997). EPNs from *Steinernematidae* family were revealed, cultivated on *Galleria mellonella* and the nematode local strains were morphologically identified. The development of EPF as the accompanying with EPN were detected to all samples. The isolates were cultivated on potato dextrose agar (PDA) and were tested on *G. mellonella* and *Tenebrio molitor larvae.* EPF was identified as genus *Beauveria* by the pathological characteristics and morphological identification. The invasive ability of local strains were studied on major pest insects of Solanaceous crops (*Tuta absoluta*, *Leptinotarsa decemlinelata, Trialeurodes vaporariorum)*. The application was carried out twice for 7 days interval and a sterile water was used in control variants. Data was analyzed with two way ANOVA (p=0.05).The average pest mortality index reached 47 % - for EPN, 63 % - for EPF. The results of preliminary investigations allow to conclude that soils in Georgia are habitation of pathogenic organisms, can be used as biocontrol agents for pest management.

Poster. **NE-15**
Cancelled

Poster. **NE-16**

Survival time and infectivity of entomopathogenic nematodes with or without pre-conditioning formulated in alginate beads

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The formulation of entomopathogenic nematodes (EPN) as infective juveniles (IJ's) in a biopesticide aims to increase their survival rate and to maintain its infectivity as a first function. To evaluate the survivorship (SU) and infectivity (IN) of IJ's formulated in alginate beads (AB), *S. glaseri* (*Sg)*, *S. carpocapsae* (Sc) and *H. bacteriophora* (Hb) were reproduced in larvae of *Galleria mellonella* and subjected to two IJ's collect treatments, namely: White traps (WT) and plaster of Paris (PP). Three days after the onset of the emergency IJ's were collected. Bi-distilled water suspension with IJ's (WS) was the control. The formulations were stored at room temperature. The counting of live and dead IJ's was performed every seven days, while IN over time was tested by exposing third instar *G. mellonella* larvae to IJ's every 7 days. The bioassays were replicated 3 times. Survival analyses was performed using Kaplan-Meier graphs and Log-rank test while infectivity analyses was evaluated by Tukey test. SU of *Sg* was 15% after 7 d and with pre-conditioning it was 23% after 17 d. *Sc* showed 87% SU after 27 d and with pre-conditioning it was 10% after 21 d. SU of pre-conditioned *Hb* was 92% after 17 d. IN of *Sg* was 15% at 7 d and at 14 d increased with pre-conditioning to 70%. After 28 d IN of *Sc* without pre-conditioning was 100% and with pre-conditioning was 55% at 21 d. IN of *Hb* at 14 d was 90% and 100% for IJ's with and without pre-conditioning, respectively. High infectivity percentages with low number of alive IJ's were observed in *Sg* and *Sc*. Thus, *Sc* without preconditioning was the formulation with best performance regarding SU and IN. Collecting *S. glaseri* IJ's on PP is advisable to promote increases in SU and IN in AB, but not in the other nematode species. Project SIP-IPN 20160475

Poster. **NE-17**

Susceptibility of Mealy Plum Aphid, *Hyalopterus Pruni* (Homoptera: Aphididae), to entomopathogenic nematodes *Steinernema carpocapsae* and *Steinernema feltiae* (Rhabditida; Steinernematidae) under laboratory conditions

Nona Mikaia; Sokhumi State University, Georgia

The mealy plum aphid (MPA), *Hyalopterus pruni* (Geoffroy) (Homoptera: Aphididae) is a pest of plum trees in Georgia. Efficacy of two entomopathogenic nematode species, *Steinernema carpocapsae* and *Steinernema feltiae*, against the adult stage of MPA was evaluated under laboratory conditions. The experiments were conducted in 10 cm petri dishes lined with a moistened filter paper. One infested Plum plant leaf containing 80-100 MPA adults was placed in each petri dish and the nematodes were applied 0, 25, 50 and 100 infective juveniles/cm^2. Plates were

incubated at 10,15 and 25°C and insect mortality was checked 72 and 96 hours after the treatment. Ten petri dishes were used for each nematode concentration and temperature. The results showed that entomopathogenic nematodes were quite virulent against MPA and the mortality was related with nematode concentration and temperature. *S. carpocapsae* species exhibited partially better results than *S. feltiae*. For both nematode species, the highest virulence was observed at 25°C and 100 IJ concentration where *S. feltiae* and *S. carpocapsae* showed 73.3% and 82.3% mortality, respectively. As conclusion, it was determined that MPA can be controlled by *S. carpocapsae* but further studies should be conducted in greenhouse and filed conditions.

Poster. **NE-18**

Temperature effects on Korean isolated entomopathogenic nematode, *Steinernema kraussei*

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This study describes the basic ecological characteristics of the entomopathogenic nematode *Steinernema kraussei* that was originally isolated from soil of Ulleung Island in Republic of Korea. The effect of temperature (15, 20, 25 and 30°C) on the infection of *Galleria mellonella* larvae by *S. kraussei* Ulleungdo strain was determined. The temperature range for infectivity was greater than that for reproduction. The optimum temperature both for infection and reproduction was 20°C. *S. kraussei* Ulleungdo strain infected the hosts even at 30°C however no progeny production occurred at 30°C. So *S. kraussei* Ulleungdo strain was better adapted to cold temperature then other Korean isolated entomopathogenic nematodes.

POSTER SESSION — Wednesday, 10:30-13:30 – ***Agnès Sorel***

Virus division

Poster. **VI-1**

A new virus from *Cotesia* parasitoid wasps fills a gap in the arthropod large dsDNA virus phylogeny

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High-throughput sequencing of *Cotesia congregata* genome revealed the presence of a viral genome distinct from the segments or the nudiviral sequences of the bracovirus. This genome is a double stranded circular DNA molecule of approximately 101.2 kb that contains 98 open reading frames and displays a 40.2% GC content. This new virus shares at least 25 genes with the baculoviruses, nudiviruses, hytrosaviruses and the newly characterized filamentous virus from *Apis mellifera*, including 11 and 13 baculovirus and nudivirus core genes, respectively. PCR amplifications showed the virus is present in all the tested tissues in both female and male adult wasps. Furthermore, partial sequences were detected in data resulting from the Illumina sequencing of the *Cotesia flavipes* genome, thus revealing such viruses might be more widespread than expected. Phylogenomic analyses based on an alignment of 37 distinct viral genes showed this new virus belongs to a clade including the hytrosaviruses and the filamentous viruses characterized in hymenopteran. Although no physical evidence are available to date, this new viral sequence might correspond to the virus-like filamentous particles observed 24 years ago in *Cotesia congregata* ovaries by Isaure de Buron and Nancy Beckage

Poster. **VI-2**

A Shannon entropy-based method to predict the localization of transmembrane proteins (BV or ODV envelopes) in the *Baculoviridae* family

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1 Universidad Nacional de Quilmes, Laboratorio de Ingeniería Genética y Biología Celular y Molecular, Bernal, Argentina; 2 Universidad Nacional de Quilmes, Laboratorio de Cronobiología, Bernal, Argentina

The family *Baculoviridae* is composed of insect-specific viruses containing covalently closed, double-stranded DNA genomes. The viral cycle presents a biphasic infection process generating progeny which express two different phenotypes: budded viruses (BVs), which are produced at the initial stage of the multiplication cycle and are responsible for the systemic infection inside the insect host; and occlusion-derived viruses (ODVs), embedded in protein crystalline occlusion bodies (OBs), which are produced in the last stage of the cycle and are required for the primary infection that takes place in the midgut epithelium cells. Both BV and ODV structures contain nucleocapsids enclosed in a lipid bilayer envelope but showing a different composition associated with the biogenesis of each viral phenotype: the cytoplasmic or nuclear membranes, respectively. Numerous viral proteins are structural and are located in one of the two membranes such as GP64 (in BVs) or the PIF complex (in ODVs), playing central roles in the cell recognition and entrance processes. Considering that lipid constitution of envelopes could correlate with differences in the transmembrane domain composition of structural viral proteins, we present a novel method based on Shannon entropy to characterize and predict the location of all transmembrane proteins of Autographa californica MNPV which is validated by proteomic studies previously reported. This work provides bioinformatics evidences to support the analyses and assists in the understanding of baculoviral protein syntax, proposing a predictive method for characterizing polypeptides encoded from baculovirus genomes.

Poster. **VI-3**

Adaptation of a Colombian Spodoptera frugiperda nucleopolyhedrovirus isolate to alternative host Heliothis virescens

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Spodoptera frugiperda multiple nucleopolyhedrovirus (SfMNPV) has a host range restricted to few insects' species, including closely related pest complexes such as *Heliothis* spp. and *Helicoverpa* spp. In a recent work, a Colombian isolate of SfMNPV (SfCOL) was used as active ingredient to develop a biopesticide to control the fall armyworm in maize, rice and cotton crops. The aim of the current study was to evaluate the SfCOL insecticidal activity against *Spodoptera frugiperda* and *Heliothis virescens* larvae after three passages of amplification over *H. virescens* larvae.

Restriction endonuclease analysis (REN) of the genomic DNA was performed using three different enzymes (*Pst* I, *Bam* HI and *Hind* III) in each amplification passage. SfCOL occlusion bodies (OBs) originally amplified over *S. frugiperda* larvae (P0) were amplified in three passages in *H. virescens* (P1, P2 and P3) by the droplet feeding method. Purified OBs suspensions were adjusted to 1 x 107OBs/mL and used for efficacy bioassay over *S. frugiperda* and *H. virescens* second instar larvae. The SfCOL (P0) efficacy against homologous insect host was 100%, while it was 76.7% against *H. virescens.* After three passages over its heterologous host (P3), SfCOL reached 100% efficacy over *H. virescens* larvae while the efficacy against *S. frugiperda* larvae diminished. These results suggest an adaptation process of SfCOL isolate over *H. virescens*, possibly due by a natural selection of certain genotypes that have better fitness than others do over *H. virescens* larvae. This study is an initial approximation for using *S. frugiperda* MNPV to control heterologous pests in different crops.

Poster. **VI-4**

Analysis of protein expression from the baculovirus AcMNPV in the cell line BTI-TN-5B1-4 at different post-infection times.

Cristina Del Rincó-Castro, Aline Palacio-Toledo, Angeles Bivián-Hernández, Mayra Chico-Andrade
University of Guanajuato, Food Department, Life Sciences Division, Campus Irapuato-Salamanca, Mexico

The correct and efficient amplification of AcMNPV baculovirus was achieved by infecting the cell line Tn5™ High Five (BTI-TN-5B1-4). The protein profile was obtained by polyacrylamide gels (SDS-PAGE) of High Five™ cell line Tn5 (BTI-TN-5B1-4) infected with AcMNPV baculovirus at different post infection times, identifying the main differences at the protein level between infected cells and uninfected. 2D PAGE polyacrylamide gels of protein profile of Tn5™ High Five cells (BTI-TN-5B1-4) infected with AcMNPV virus was obtained, which allowed selecting representative differentially expressed proteins in infected and uninfected cells. Was obtained the protein sequence of each protein induced by AcMNPV virus infection in BTI-TN- 5B1-4 cells and those expressed in the uninfected cells, but whose expression was repressed by the virus. The sample is placed on the plate using six fold as a-cyano matrix and analyzed in a MALDI TOF/TOF 4800. With the MS/MS spectra obtained a search performed with the search algorithm ProteinPilot Paragon's software with a percentage of confidence of 66%. It was observed that as the viral infection progressed, the amount of protein increased in the infected cells, while this amount decreased in uninfected cells. We hope these results allow even understand a small scale, some of the defense mechanisms of healthy cells as well as the ability of the virus to turn off genes of cellular metabolism and those affecting the cellular structure, all in order to express different viral proteins that improve and facilitate the infection and spread from the AcMNPV virus. An important contribution of this work was the information about which proteins are induced or repressed by AcMNPV virus in insect cells growing *in vitro*.

Poster. **VI-5**

Baculovirus infection induces disassembly of nuclear lamina

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The nuclear lamina presents a substantial obstacle against those DNA viruses which do not disrupt nuclear membranes for their release. For herpesviruses, nuclear lamina is dissolved through a kinase-mediated phosphorylation mechanism before viral nucleocapsids engage the inner nuclear membrane to bud into the perinuclear space. The nucleocapsids of baculoviruses are proposed to egress from the nucleus by budding at the nuclear membrane, but whether the nuclear lamina undergoes disassembly during baculovirus infection remains unknown. In this report, we generated a stable Sf9 cell line expressing GFP-tagged lamin B. In the interphase nucleus GFP-lamin B fluorescence exhibited a perinuclear localization pattern, suggesting that GFP-lamin B was incorporated into the nuclear lamina. We then investigated the effect of baculovirus infection on nuclear lamina with this cell line. The results showed that the amount of GFP-lamin B in virus-infected cells remained constant during viral infection. GFP-lamin B was distributed into the nucleus and associated with the edge of the electron-dense matte region of the virogenic stoma, intranuclear microvesicles, nucleocapsid and envelope of occlusion derived virions (ODV) in virus-infected cells, while it was specifically localized to the nuclear rim in mock-infected cells. We also found that the phosphorylation of GFP-lamin B increased during baculovirus infection. Our observations indicated that baculovirus infection induces phosphorylation of lamin B and partial disassembly of nuclear lamina in Sf9 cells.

Poster. **VI-6-STU**

Baculovirus ODV occluded by polyhedra in different insect cell lines

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Baculoviruses are insect pathogenic viruses with circular, double-stranded DNA genomes that range in size from 80 to 180 kilobase pair (kbp). Two distinct virion phenotypes are produced in its replication cycle. Initially, capsids bud from virus-infected cells to form budded virions (BV). Subsequently, capsids remain with the nucleus, become enveloped and are then packaged within occlusion bodies. This form of the virus particle is frequently known as occlusion-derived virus (ODV). The occlusion bodies are responsible for virus stability outside the host insect and for delivering infectious virus (ODV) to insect midgut cells. In order to investigate factors involved in the packaging of ODV by polyhedra different insect cell lines *Spodoptera frugiperda* (Sf9) and *Trichoplusia ni* (*T.ni*) were infected with wild type Autographa californica multicapsid nucleopolyhedrovirus (AcMNPV) and a recombinant virus (rAcMNPV), which lacked p26, p10 and p74 genes. Polyhedra were isolated from both cell lines, dissolved in an alkaline buffer to release the ODV, which were then purified on continuous sucrose density gradients. ODV could be readily isolated from both AcMNPV and rAcMNPV amplified in *T.ni* cells but not from viruses propagated in Sf9 cells owing to much lower yields of polyhedra. Therefore, AcMNPV and rAcMNPV polyhedra from Sf9 cells were examined using transmission electron microscopy (TEM). These results showed Sf9-derived AcMNPV packaged many more ODV than rAcMNPV. In addition, qPCR was used to show that the virus DNA content of AcMNPV polyhedra was 7.8 – fold higher than that of rAcMNPV occlusion bodies. These results show that there is a differential effect on ODV packaging of the triple gene deletion (p26, p10 and p74) in Sf9 and T.ni cells.

Poster. **VI-7-STU**

Biochemical characterisation of the baculovirus *per os* infectivity complex

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Baculoviruses infect insect midgut epithelial cells via the occlusion-derived form of virus (ODVs). For *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) at least seven conserved ODV envelope proteins are essential for oral infectivity and are called *per os* infectivity factors (PIFs). Previous work showed that most of these PIF's are present in a multi-molecular complex. PIF1, PIF2 and PIF3 are essential for complex formation and PIF4 is important for stabilization of this complex. These four PIF's are considered to be the core components of the PIF-complex. Another PIF-protein, P74, has been identified as interaction partner of PIF1 and is more loosely associated to the complex. Analysis of larvae-derived ODV's (L-ODV) showed clear biochemical differences when compared with the cell culture-derived ODV's (C-ODV). On western blots with L-ODV's, the band representing the PIF-complex is less well defined and migrates faster than with C-ODV's. Moreover, in C-ODV's, PIF1 monomers were detected together with the complex while these were absent in L-ODV's. These observations suggest that the composition, stoichiometry and/or conformation of the complex might be different depending on the origin of the virus. Furthermore, P74 has been shown to be cleaved only in L- ODV's. This cleavage event is mediated by a still-to-be-identified endogenous protease, which is co-occluded with the L-ODV's. Together, these observations suggest that the PIF-complex and interaction partners have different characteristics, depending on whether the virus is propagated in insect larvae or cultured cells.

Poster. **VI-8-STU**

Characterization of VP91 of Helicoverpa armigera nucleopolyhedrovirus

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VP91 is one of the 37 core proteins present in all sequenced baculoviruses. It was identified as an ODV envelope protein and a component of the *per os* infectivity factor (PIF) complex. Recent study suggested that VP91 of *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) was required for both nucleocapsid assembly and oral infection. In the present study, *ha76,* the gene that encodes the VP91 homologue of *Helicoverpa amigera* nucleopolyhedrovirus (HearNPV) was characterized. Transcription and expression of *ha76* suggest that *ha76 i* s a late viral gene. Immunofluorescence assays showed that HA76 localized to the ring zone region within the nucleus of HearNPV infected cells. The N-terminal 30 amino acids (aa) of HA76, carrying a putative inner nuclear membrane localization signal, were competent of targeting of EGFP protein to the nuclear envelope. To better understand the function of VP91, *ha76* deleted and repaired viruses were generated. Transfection-infection assays and electron microscopy (EM) showed that *ha76* was required for budded virus (BV) propagation and nucleocapsid formation, which was similar to the phenotype of AcMNPV VP91. Truncation of the N- or C-terminus of HA76 identified that a protein with the N-terminal 599 aa (1-599aa), but not 405 aa (1-405aa), was able to rescue BV infectivity of *ha76* -null HearNPV. Further truncational analysis showed that the middle domain (447-599 aa) of HA76 could partially rescue the ability of *ha76* -null HearNPV to produce infectious BV, albeit the BV titer decreased by a ˜100 fold. EM analysis on these recombinants is ongoing to explore the role of HA76 on nucleocapsid and ODV morphogenesis.

Poster. **VI-9**

Co-infection by SeIV iflavirus and Spodoptera exigua multiple nucleopolyhedrovirus (SeMNPV): effects on occlusion body structure and conformation

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Lepidopteran populations frequently harbor cryptic infections involving one or more viruses. Three species have been repeatedly identified in covertly infected *Spodoptera exigua* : the *S. exigua multiple nucleopolyhedrovirus* (SeMNPV), the iflaviruses SeIV1 and SeIV2. Mixed infections have been detected in laboratory colonies and field collected insects but little is known about the interaction between these viruses and their effects on the insecticidal properties of SeMNPV occlusion bodies (OBs). When SeMNPV and SeIV were inoculated in the same host and a productive baculovirus infection occurred, PCR-based techniques and electron microcopy (TEM) of OBs previously indicated contamination of OBs by iflavirus particles (Jakubowska et al 2016, PeerJ e1687). In the present study OB populations generated by co-inoculation of SeM- NPV and one of three iflavirus treatments: SeIV1, SeIV2, and a 1:1 mixture of SeIV1+SeIV2 were examined for OB structure and iflavirus load, in comparison to OB populations from iflavirus-free insects. OB populations were purified using a continuous sucrose gradient. Interestingly, two OB bands were observed and collected for the three iflavirus treatments whereas only one band was present in the SeMNPV control. RNA was extracted from OBs and specific viral loads quantified by qPCR for the fragmented OB population corresponding to the upper and low bands collected. These results allowed us to correlate the different morphological OB conformation with high SeIV1 loads. We conclude that SeIV1 co-infection altered OB conformation that might explain the biological properties of these OBs. Results of ongoing experiments will be reported aimed at determining OB physical characteristics by TEM.

Poster. **VI-10-STU**

Comparison of genome replication rates of fast-killing versus slow-killing SfAV isolates

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Ascoviruses (AVs) are double-stranded DNA viruses with circular genomes. They primarily attack lepidopteran larvae and pupae. AVs are transmitted by female parasitic wasps via oviposition and cause a chronic disease that terminates mortality. It is likely that a prolonged infectious period results in a higher rate of virus transmission among susceptible populations by parasitoids. Recently, we isolated an atypical AV, Spodoptera frugiperda ascovirus-X (SfAV-X) that kills host larvae rapidly. In this study, the speed of mortality induced by SfAV-X was compared with a slow-killing AV (SfAV-F) to elucidate factors that influence their speed of kill. The mean killing speed of SfAV-X was 2.7 days, while that of SfAV-F was 22.0 days. We hypothesized that the higher killing speed of SfAV-X was due to a higher rate of replication of its genomic DNA when compared with SfAV-F. The rate of viral DNA replication was measured by qPCR using oligomers to amplify the major capsid protein gene of each virus, and growth parameters were compared using Gompertz's growth curve for both strains. Our results showed that the rate of SfAV-X DNA replication was significantly higher than that of SfAV-F. Although the time required for viral DNA replication to reach a plateau (Tmax) was significantly shorter for SfAV-X than for SfAV-F, the difference in Tmax was only 2.2 days. By comparing this difference with that of the killing speed of 19.3 days, we conclude that viral replication rate is not the direct cause for the difference in killing speed of the two strains. Other possible factors associated with killing speed will be discussed.

Poster. **VI-11**

Development of a highly efficient recombinant system for Anticarsia gemmatalis multiple nucleopolyhedrovirus (AgMNPV) and findings about baculoviral replication in absence of the essential gene orf1629

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Anticarsia gemmatalis multiple nucleopolyhedronvirus (AgMNPV) is one of the most extensively used viral pesticides, but its performance as a biological control agent in more temperate climates requires the improvement of its biopesticidal properties. Genetic modification is a powerful strategy to improve these properties in AgMNPV including heterologous genes in the viral DNA. In our laboratory, we have previously developed a homologous recombination (HR) system to modify AgMNPV genome based on HR in cultured insect cells. Nevertheless, recombinant AgMNPV isolation has proven to be time-consuming. In order to simplify this procedure, we developed a novel recombination system based on a defective AgMNPV (AgMNPV-Δ1629) lacking an essential gene (*orf 1629)* that replicates in a transgenic insect cell line expressing this gene (UFLAg286-1629). By co-transfection of non-transgenic cells (UFLAg286) with parental AgMNPV-Δ1629 DNA and a transfer plasmid (containing *orf1629)*, recombinant baculoviruses are recovered. HR restoring *orf 1629* and allows insertion of pesticidal genes and/or a marker genes under the control of strong viral promoters. We constructed AgMNPV-Δ1629 and demonstrated the complementation capacity of the transgenic cell line. After this, we tested the system by isolating a recombinant virus carrying the green fluorescent protein gene. In addition, novel results were found about the or*f1629* gene indicating that baculoviral DNA replicates efficiently in absence of this essential gene and that occlusion bodies appear in transfected cells and confirmed that this gene is essential for budded virus formation.

Poster. **VI-12**

Does AcMNPV loose its generalist potential when adapting to a specific host?

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A generalist virus, such as *Autographa californica multiple Nucleopolyhedrovirus* (AcMNPV), is able to replicate in many host species. This capacity might result from different levels of genetic variation, the maintenance of which is facilitated by the transmission of MNPVs as occlusion bodies (OBs) enclosing many virions each containing several genomes. Upon selection on a particular type of host, the baculovirus population should evolve to increase its fitness in this species. The question is whether this specialisation comes at the cost on the generalist potential of the virus. To understand if AcMNPV could draw on standing genetic variation to maintain its generalist potential, we undertook an experimental evolution protocol. A highly polymorphic AcMNPV strain was passaged 10 times in *Trichoplusia ni* or *Spodotera exigua* in 10 replicates. The last generation of virus produced was tested with bioassay on 4 host species, including resistant species. Fitness in different hosts was measured as the production of OBs. We found that the viral populations evolved to the semi-permissive host *S exigua* had lost more of their generalist potential than those evolved on *T ni.*

Poster. **VI-13**

Earthworm mediated dispersal of baculovirus occlusion bodies in soil

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The soil is indisputably the most important reservoir of baculovirus occlusion bodies (OB) in the environment. Despite this, the dynamics of OB movement between soil and plant surfaces, and *vice versa* have been poorly studied, with some important exceptions in the studies performed by James Fuxa and colleagues in Louisiana. Ever since the pioneering studies by Darwin, the role of earthworms in the breakdown of organic matter, nutrient cycling and the redistribution of soil particles has been recognized as a key contribution to defining soil texture, aeration, drainage, microbiota and fertility. In the present study we demonstrate that earthworms (*Eisenia fetida)* were capable of moving OBs of Spodoptera frugiperda MNPV from the surface of laboratory soil microcosms to a depth of up to 22-24 cm over a 15 day period. Incubation of earthworms for 1 week in artificial soil containing 1 x 10e9 OBs did not result in a significant loss of OB pathogenicity as determined by soil-diet incorporation bioassay in *S. frugiperda* larvae. The earthworm gut was found to have a slightly acid pH using acid-base pH indicators. A field study indicated that

earthworms were capable of moving OBs from the soil surface to a depth of 8 cm, although positive results were only observed in one out of five replicates. We conclude that earthworms could contribute to the dynamics of baculovirus OB populations in soils. As such, earthworms deserve further study for their role in enhancing the dispersal and persistence of soil OB populations.

Poster moved to contributed papers MC2. **VI-14-STU**

Genetic and biological characterisation of a novel alphabaculovirus for the microbial control of *Cryptophlebia peltastica*

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Cryptophlebia peltastica (Meyrick) (Lepidoptera: Tortricidae) is an economically damaging pest of litchis and macadamias in South Africa. An IPM programme has been developed for the control of *C. peltastica*, however it has not yet provided satisfactory control of this pest. A baculovirus for use as a microbial control agent has not yet been considered. A laboratory culture of *C. peltastica* was established and maintained and larvae showing symptoms of viral infection were collected and examined for the presence of a baculovirus. An alphabaculovirus (SNPV) was morphologically identified using purified OBs that were sectioned and observed by transmission electron microscopy. The NPV was characterised using PCR and a BLAST analysis. A 93% similarity to a partial polH gene sequence from *Epinotia granitalis* was found. REN profiles were generated for the SNPV, but could not be compared to other profiles. An 116646 bp whole genome was sequenced with a GC content of 37.1% and 105 ORFs. Bioassays were used to determine the virulence of the NPV against *C. peltastica*, *Thaumatotibia leucotreta* and *Cydia pomonella*. The NPV was more virulent against *T. leucotreta* and *C. pomonella* than its homologous host. This is the first report of a novel SNPV isolated from *C. peltastica*. Further research will focus on mass producing the virus and determining the efficacy in the field.

Poster. **VI-15**

Genomic Analysis of Four Plutella xylostella Granulovirus Isolates

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Diamondback moth (*Plutella xyslostella)* is considered the most destructive insect pest of cruciferous crops worldwide for which control is estimated to cost up to five billion US dollars annually. Plutella xylostella granulovirus (PxGV) is a highly virulent pathogen that has co- evolved with diamondback moth and is considered a potential biopesticide. We sequenced four isolates of PxGV from Taiwan, China and Malaysia using the Illumina NextSeq 500 platform resulting in 13,186 to 23,878 X coverage. All four genomes share 99.9% sequence homology and 40.7% GC content. Sequence variability is greatest within the four homologous repeat regions which exhibit 59.8% pairwise identity. All four genomes encode two fewer putative ORFs in conserved order compared to the RefSeq Japanese isolate (NC 002593). Phylogenetic analysis shows the Japanese isolate is more homologous to a Taiwan isolate while the Chinese isolate is most divergent. Genomes of PxGV isolates share very high sequence conservation and order despite geographical separation.

Poster. **VI-16**

Impact of single and multiple morphotypes on genome-wide selection in baculovirus

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The baculoviruses are a group of insect viruses with large dsDNA genomes, and their primary infection is triggered by rod-shaped enveloped virions embedded in a crystalline protein matrix. Each virion may contain one (SNPV) or more nucleocapsid (MNPV) within an envelope. At the primary MNPV host infection, multiple genomes copies enter the cell simultaneously, implying an obligatory co-infection in the initial infection step and also an oscillatory pattern in the effective population size through time. While in SNPV each virion has just one genome and the likelihood of co-infection in the first round of infection is lower. Therefore, it is reasonable to assume that the multiplicity of infection is distinct for these morphotypes. Therefore, we investigate the impact of these two morphotypes on baculoviruses evolution using available genomic data sampled both at the intraspecific and interspecific level. We estimated the dN/dS for each gene and we focused on the genome-wide patterns instead of on individual genes. At the intraspecific level, our analysis shows that the MNPVs have a more heterogeneous selection profiles than the SNPVs. However, at the interspecific level no differences in the selection profiles were observed for the two phenotypes. Taken together, these results suggest that the heterogeneity observed at the intraspecific dataset may be the result of a differential response time to selection rather than distinct selection coefficients. Actually, several studies have shown that viral co-infection may increase the time to purge slightly deleterious mutations. This is the first evidence of the impact of nucleocapsid aggregation on the genome-wide selective patterns in baculoviruses.

Poster. **VI-17-STU**

Improving Baculovirus Surface Display System

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Baculoviruses have been widely used for recombinant protein expression for a long time and recently this has been taken a step forward by the pseudotyping approach, which is the display of recombinant proteins on the budded virus envelope. Pseudotyped baculoviruses could serve as safe vaccines by displaying the antigenic epitopes of a target virus protein. They also have the potential to be used as tools in diagnostic assays by mimicking lethal viruses in safer environments. In order to pseudotype budded viruses, the gene of interest is inserted into the baculovirus genome in frame between the GP64 signal peptide and the transmembrane domain (TMD) under the control of an insect promoter. In this project, fusing the recombinant proteins to either the full length or truncated GP64 TMD was compared with the aim of improving the baculovirus surface display system. It has been shown that, truncating the GP64 TMD by 1382 bp from the amino terminus, improves the expression levels of the recombinant

protein. In addition, recombinant baculovirus with a truncated GP64 TMD was observed to have an enhanced infectivity, especially in TnHi5 cell culture, compared to recombinant viruses containing the full length GP64 TMD. Furthermore, the expression levels of several proteins were greatly improved by fusing them to the truncated GP64 TMD, which is possibly due to the increased stability and solubility achieved from the fusion.

Poster. **VI-18**

Insect immune system to determine baculoviruses host specificity

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Baculoviruses are insect-specific DNA viruses with restricted host range, and serve as viral vectors for bioindustry applications such as foreign gene expression, vaccine production, and pest control. *Autographa californica* nucleopolyhedrovirus (AcMNPV), a prototype of a commercially available and widely used baculovirus, can infect 39 species in 13 families. *Bombyx mori* nucleopolyhedrovirus (BmNPV) is a major pathogen of silkworms and has developed high host specificity to *Bombyx mori*. Interestingly, on a genomic level, the AcMNPV and BmNPV are highly homologous, but they share no overlapping host range. These two quite similar viruses have extremely different infection outcomes in *Bombyx mori*. We theorize that the determination of host specificity may depend on virus-host interactions, and that several genes may be involved in determining host specificity. Therefore, we used next-generation sequencing (NGS) to analyze the transcriptome response of the hosts to these viruses. The transcriptome library was constructed, annotated, and grouped after sequence assembly. A comparison of gene expressions shows several significant differences in the gene expression profiles of BmNPV and AcMNPV, especially in cases where genes involved in immune responses are verified by RNA interference. The manipulation of virus-host specificity could provide a breakthrough for the application of baculovirus in protein expression systems and in the development of bio-control agents.

Poster. **VI-19-STU**

Insecticidal activity of Phthorimaea operculella granulovirus isolates from Southern Europe on *Tuta absoluta*

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The tomato leafminer moth, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is a key pest of tomato originating from Chile that invaded Spain in 2006 and quickly spread throughout Europe, the Middle East and Africa. Tomato is the preferred host plant, but larvae also attack other crop plants of the nightshade family, including potato, eggplant and cucumber. The inefficiency of chemical insecticides due to both resistance and the concealing behavior of the pest have made growers increase pesticide rates and number of applications, leading to higher environmental impact and to increases of up to 70% of total pest management costs in many instances. Therefore, more effective, inexpensive and environmentally safe control techniques are needed. Biological control based on the use of entomophathogens such as baculoviruses has demonstrated to comply with these requirements in many other crop-pest complexes. A granulovirus isolated from *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) is effective agains *T. absoluta* larvae. This work aimed at comparing the insecticidal performance on *T. absoluta* larvae of different granuloviruses isolated from *P. operculella* in Spain, Greece and Italy. Bioassays to determine pathogenicity, expressed as mean lethal concentration (LC50), virulence, expressed as mean time to death (MTD) and productivity, expressed as OBs per larvae, were carried out on L2-L3 *T. absoluta* larvae using tomato leaf discs.

Poster. **VI-20-STU**

Insecticidal evaluation of a recombinant Trichoplusia ni granulovirus (TnGV) generated by biolistics

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Recombinant baculoviruses have been developed almost exclusively from the Alfabaculovirus genus, mostly from the AcNPV. The generation of recombinant baculoviruses requires a permissible cell line to perform the co-transfection *in vitro*. The great majority of baculoviruses are impeded to develop a recombinant strain due to this limitation. Previous work in our lab made possible to obtain a recombinant granulovirus by co-transfecting larvae using biolistics, which opened the possibility to obtain, theoretically, a recombinant strain of any baculovirus species. This report shows the evaluation of two recombinant TnGVs strains obtained by microparticle bombardment, integrating genes for the Cn10 toxin from the scorpion *Centruroides noxius* and the Cyt1Aa toxin from *Bacillus thuringiensis* svar. *israelensis*, under the control of the polyhedrin and p10 promoters, respectively. Additionally, specific vectors were developed to integrate the constructs within the *p49* gene of TnGV, which is involved in the suppression response to apoptosis in the host cells. After the recombinant strains were detected by the expression of the GFP protein, included in both constructs, and a series of purification cycles, bioassays against first instar *T. ni* larvae were performed. A concentration of 1,600 OB/mm2 of the TnGV-*p49* ::Cn10 and TnGV-*p49* ::cyt constructs was used to estimate LT50s of 7 and 4.5 days, respectively, compared to 15 days estimated for the wild type strain. All the control individuals reached pupation. LD50s were also estimated for both constructs after 8 days postinfection, estimating 620 OB/mm2 for the TnGV-*p49* cyt construct, significantly different to the other construct and the wild-type strain, which showed no difference.

Poster. **VI-21-STU**

Is ORF1629 essential or not for the replication of baculovirus?

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Recombinant baculovirus genome that lacks part of ORF1629 has been used to improve efficiency of recombinant virus selection because a functional ORF1629 is believed to essential for viral replication in insect cells. It can be rescued/restored by recombination with a transfer vector containing an intact entire ORF1629. Several baculovirus recombinant bacmids were generated for this purpose. Recombinant bacmid ApGOZA, one

of them, has a mini-F replicon for *Escherichia coli* and the truncated ORF1629 gene. Accidently, we could observe the replication of ApGOZA alone in Sf9 cells. Produced virus from ApGOZA was analyzed for the existence of truncated ORF1629 not intact ORF1629 by PCR amplification and genomic sequencing. To investigate the influence of incomplete ORF1629 to the replication of virus, the viral growth, BV production and polyhedral formation were analyzed comparing to wild type virus. The results suggested the possibility that ORF1629 is not essential for viral replication in insect cells.

Poster. **VI-22-STU**

Light at a fixed time period after infection is needed for Spodoptera exigua MNPV-induced tree-top disease

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Host behavioural manipulation is a common strategy used by parasites to enhance their survival and/or transmission. Baculoviruses induce hypermobility and tree-top disease (pre-death climbing behaviour) in their caterpillar hosts. However, little is known about the underlying mechanisms of this behavioural manipulation. Previously, we showed that the baculovirus Spodoptera exigua multiple nucleopolyhedrovirus (SeMNPV) induced tree-top disease at 3 days post infection (dpi) in *S. exigua* larvae and that light from above plays a key role in triggering this behaviour. Here we further studied the role of light in this process. We found that light was needed between 43-50 hours post infection (hpi) to induce tree-top disease: infected larvae that were not exposed to light in this period finally died at low positions. We also showed that light before this period did not affect the final positions where the larvae died. Overall, light in a particular time frame at 2 dpi is needed to trigger SeMNPV-induced tree-top disease in *S. exigua* larvae.

Poster. **VI-23-STU**

Morphological properties of the occlusion body of Adoxophyes orana granulovirus

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In 1999, we found a granulovirus (GV) that produced an unusual morphology of occlusion bodies (OBs) in an *Adoxophyes* sp. (Lepidoptera: Tortricidae) larva in a tea field in Miyazaki Prefecture, Japan. This isolate is considered to be a mutant of Adoxophyes orana granulovirus, AdorGV (AdorGV-M), because 99.7% of the nucleotide sequence of AdorGV-M is identical to that of an English isolate of AdorGV, AdorGV-E. AdorGV-E OBs have a typical ovo-cylindrical shape, with a diameter of 0.3-0.5 μm, and each OB contains one virion. On the other hand, AdorGV-M has cuboidal-shaped OBs whose diameter is 0.5-2.0 μm, and the number of virions per OB is unknown. In this study, we quantified viral DNA in OBs of both AdorGV-E and -M, and compared the amounts to reveal the number of virions contained in an OB of AdorGV-M. The result showed that the two isolates had the same quantity of viral DNA in their OBs, and thus confirmed that one OB of AdorGV-M has one virion. To reveal time-dependent formation of OB shape and size, the fat bodies of *A. homai* larvae inoculated with both strains were observed in a time course by transmission electron microscopy, and OB sizes were measured from micrographs. Each isolate displayed a different process of OB formation. In AdorGV-E, the virions began to be covered from one side with an ovo-cylindrical OB at 96 hours post- inoculation (hpi), and most of them were completely occluded at 120 hpi. In AdorGV-M, the virions began to be occluded at 96 hpi, as with AdorGV-E; however, the virions were occluded with a cuboidal-shaped OB. Moreover, the OB size of AdorGV-M was similar to that of AdorGV- E at 120 hpi, but continued to grow until 192 hpi.

Poster. **VI-24**

New Polydnavirus genomes of *Microgaster* wasps

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Braconidae parasitoid wasps display one of the largest species richness on earth, with several dozen of thousands of species. The presence of a virus-derived gene delivery weapon – known as polydnavirus (PDV) – integrated in the wasp's genome since at least 100 My could explain this evolutionary success. PDVs play a key-role in parasitism by manipulating the immune system of lepidopteran hosts, but also by acting on the behavior of host choice for egg-laying. Wild *Microgaster subcompletus*, *M. nixalebion* and *M. alebion* were chosen for a comparative genomic approach since they are closely-related species, but displaying extremely distinct parasitism strategies concerning host species and host ranges (generalists vs. specialists). *Microgaster* wasps are furthermore genetically distant to other wasps with known PDV genomes (eg. *Cotesia*, *Glyptapanteles*, *Microplitis*). Viral circles isolated from ovarian tissues of the 3
Microgaster species were sequenced using Illumina technology. Paired-end reads were cleaned and assembled using Newbler and Trinity software. Ab initio gene models were predicted using Augustus and FGenesh softwares, confirmed using BLAST search with known PDV protein databases and curated manually for *M. subcompletus*. In this species (MsBV), 383kb were assembled in 35 contigs. Assembly displayed a N50 length of 12.3kb, and is formed by 12 putative circles and 23 incomplete fragments. A total of 204 genes were annotated, among them 13 were pseudogenes and 25 were putative new PDV genes. Both gene content, number of genomic fragments and total genome size of MsBV are similar to other sequenced PDVs, suggesting a nearly-exhaustive dataset for *Microgaster subcompletus*, now to be compared to the other *Microgaster* PDV genomes.

Poster. **VI-25-STU**

Novel Cydia pomonella granulovirus isolates break virus resistance in codling moth

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Cydia pomonella granulovirus (CpGV) (family *Baculoviridae)* is an effective viral agent to control codling moth (CM, *Cydia pomonella* L.) populations in apple and pear plantations. Since 2005, resistance of CM field populations against CpGV products has been documented in more than 40 orchards and two different types of resistance of CM (sex-linked and autosomal inheritance) had been successively found in Europe. Most resistant CM populations can be controlled by newly registered, resistance breaking CpGV isolates but some resistant CM populations are still difficult to be controlled. Therefore, searching for other resistance breaking CpGV isolates is of urgent need. We determined the efficacy of seven Chinese CpGV isolates on different CM laboratory strains, a sensitive and two resistant CM strains, in bioassays. Among them, two isolates (CpGV-JQ, CpGV-ZY2) showed virulence comparable to already described CpGV isolates. Interestingly, two isolates (CpGV-ZY, CpGV-WW) were more infective to the autosomal inherited resistant CM strain than to the sex-linked resistant strain and differed in this regard from any previously recorded CpGV isolate. All isolates were sequenced using next generation sequencing and were grouped into previously reported CpGV genome types A to E. In depth genome sequence comparisons are going on to identify the genetic factors determining the CpGV virulence in different CM strains. These studies will allow us to unveil the partial resistance mechanism and point the way to improve novel strategies for resistance management. This research is supported by a grant of the China Scholarship Council.

Poster. **VI-26**

Nuclear translocation signal of AcMNPV ME53 influences overall virus production while the Zn finger is important for virus production early in infection

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me53, one of the major immediate early genes expressed immediately following baculovirus infection, is highly conserved in all sequenced lepidopteran alpha- and beta-baculoviruses including Autographa californica nucleopolyhedrovirus (AcMNPV) in the family *Baculoviridae*. Although AcMNPV ME53 is not essential for virus DNA replication, its deletion compromises budded virus production to 0.01% of wild type. The ME53 polypeptide (449 amino acid) contains a nuclear translocation sequence (NTS) at the N-terminus, and a conserved putative C4 zinc finger motif (ZnF) at the C-terminus. C4 zinc fingers are often associated with transcription factors. The nuclear localization of ME53 and its requirement for optimal budded virus production suggest that it may play a role in transcriptional regulation and virus assembly. Therefore, qRT-PCR and viral growth curves were used to determine the importance of NTS or ZnF on viral gene transcript levels and assembly. Deletion of the NTS resulted in a reduction of virus production to 0.01%, comparable to the reduced yield seen for a ME53 KO. However, deletion of the ZnF did not significantly increase or decrease viral gene transcript levels (not more than 2.5 fold). However, the absence of ZnF did compromise budded virus production at early times post transfection. These findings suggest that ME53 may play a complex role in virus assembly/production rather than in regulation of viral gene transcription.

Poster. **VI-27-STU**

Overview of bee viruses in wild hymenoptera

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The Colony Collapsed Disorder (CCD) is a multifactorial syndrome, which can affect honeybees in the entire world and where interactions between pesticides and pathogens play an important role. Many pathogens frequently infect bees including more than twenty viruses. We have found a honeybee virus had jumped into ants, which suggests that wild pollinating bees and ants could serve as reservoir to some bee viruses. We were thus interested in the prevalence of bee viruses in a large range of wild pollinating bees and ants. We sampled 330 ants and 250 wild bees occurring in sympatry in 50 localities in Western & Eastern Canada, French Guyana and France. All samples were individually placed in RNA later before total RNA extraction and RT-PCR were performed. Taxonomic determination of the hymenopteran host species was performed using cDNA barcoding, whereby the Cytochrome Oxidase I (COI) of the insect were sequenced. Then, we used degenerated primers for the detection of *Lake Sinai Virus* (LSV) and the newly discovered Halictus scabiosae Associated Virus (HsAV – Bigot et al, unpublished data). Furthermore, a multiplex PCR detection of another 6 ssRNA+ bee viruses (CBPV, ABPV, DWV, SBV, IAPV, and BQCV) was performed. PCR detection showed at least 4 LSV positives samples from a new locality and the first *in vivo* detection of HsAV, which had only been described in silico so far. This study brings an overview of the prevalence of honeybee- infecting viruses on a large number of wild bees, and will help at determining the ecological role of the presence of bee viruses in ants. Altogether, our study establishes a first step in the understanding of the ecological network in which bee viruses might evolve.

Poster. **VI-28**

Polydnavirus-Encoded MicroRNA exerts different effects on the immune responses in *Spodoptera litura* (Fabricius) and *Snellenius manilae* (Ashmead)

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Innate immune responses are the most significant defense against pathogen infections. Antimicrobial responses, Toll pathways, produce different kinds of antimicrobial peptides (AMPs) to protect insects when triggered, but Polydnaviruses (PDVs) can suppress these kinds of immune reactions. PDVs are symbiotic viruses used by parasitoid wasps to manipulate the physiology of hosts and induce parasitism. Virus particles are only produced

by calyx cells in female wasp ovaries and enter hosts alongside wasp eggs. Viral products are essential for wasp development and ensure wasp survival in host larvae by interfering with the immune responses and development of hosts. The proviral genome is comprised of two components, the core gene and encapsidated genome, which produce gene products for replication and virulence. Encapsidated genomes are the main weapons used to suppress immune responses as they not only decrease gene expression, but also reduce AMPs from the host. The genome is made up of 15 segments, which are individually packaged into virions. The noncoding regions of the PDVs genome are responsible for immunosuppression. MicroRNAs (miRNAs) are small noncoding RNAs that play a key role in regulating gene expression in eukaryotes. The encapsidated genome have been found to persist in host genomes through integration and can express several miRNAs through structural prediction. It is theorized that these predicted miRNAs are able to suppress the immune responses of *Spodoptera litura*. This study seeks to explore the novel roles of miRNAs in the immunosuppression of PDV hosts and the mechanisms underlying this phenomenon. Combined with other control agents, this unique feature may hold potential for pest management control in the future.

Poster. **VI-29**

Role of lef-5 from SeMNPV in the stability of baculovirus in cell culture

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One of the major problems for baculovirus production in cell culture is the rapid generation of defective viruses. Transcriptional studies of the *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) infective process reveled a different expression pattern when virus was replicating in *in vitro* or *in vivo* conditions. Expression of the late expression factor 5 (*lef-5)* was found to be significantly reduced when virus was replicating in Se301 cells, in comparison when virus was replicating in *S. exigua* larvae. From this observation arises the idea that lef-5 activity could be related with the baculovirus stability when multiplied in insect cells. To test this hypothesis, recombinant *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV) expressing *lef-5* gene from SeMNPV were generated and the viral stability was monitored during successive infection passages in Sf21 insect cells by evaluating the presence of the GFP reporter in the viral genome. The control viruses showed a loss of ability to express GFP faster than those viruses expressing *lef-5* although, the replication ability was the same for both viruses. These results support the influence of lef-5 in viral stability during the multiplication process.

Poster. **VI-30-STU**

Spodoptera exigua iflavirus co-inoculation alters the insecticidal properties of Spodoptera exigua multiple nucleopolyhedrovirus (SeMNPV) occlusion bodies

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The *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) is a specific pathogen that can efficiently control this pest in greenhouse crops in southern Spain and elsewhere. Studies on persistent sublethal infections of *S. exigua* insect colonies revealed the presence of RNA viruses identified as two iflaviruses species (SeIV1 and SeIV2). SeMNPV and SeIV1/2 could be detected in both field and laboratory insect populations using PCR-based techniques. In this study, we evaluated the effect of SeIV1/2 co-infection on the insecticidal characteristics of SeMNPV, including pathogenicity, virulence and transmission traits. Bioassays were performed by simultaneously inoculating second instars with one of five SeMNPV occlusion body (OB) concentrations and one of the following four treatments: i) SeIV1; ii) SeIV2; iii) SeIV1+SeIV2; and iv) mock-infected control. Iflavirus co-inoculation consistently reduced SeMNPV median lethal concentrations (LC50) for *S. exigua* larvae compared to those infected with the SeMNPV alone. The speed of kill of SeMNPV was similar in the presence or absence of the iflaviruses. Conversely, OB production upon death was significantly reduced due to a reduction in weight gain in iflavirus infected insects. Adults survivors of a sublethal SeMNPV OB treatment were examined for covert infection. SeMNPV DNA was found to be present at a high prevalence in all treatments (70-89 %), but viral load was quantified significantly less abundant in adults that had been previously co-inoculated with SeIV1. In conclusion, iflavirus co-infection increased the pathogenicity of SeMNPV OBs. We predict that SeMNPV-SeIV interactions might reduce insect-to-insect horizontal transmission under field conditions

Poster. **VI-31**

Spodoptera frugiperda granulovirus: genomic organization of an Argentinean isolate

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The fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) is a major maize crop pest. Its chemical control has been impaired due to the emergence of resistance. Therefore, the application of different control alternatives is of great concern. The use of baculoviruses as biological control agents is an interesting tool as it shows a very low risk of resistance appearance. Baculoviruses that infect the fall armyworm have been described. SfMNPV belonging to the Alphabaculovirus genus, with several isolates reported; and SfGV, a Betabaculovirus with at least two isolates reported from Colombia and Brazil, one of them with its complete genome described (Colombian SfGV, VG008). Here we describe an Argentinean isolate of SfGV, SfGV- ARG. SfGV-ARG was replicated in *S. frugiperda* larvae. Occlusion bodies (OBs) were used for protein profile analysis and DNA purification. Its genome was sequenced by an Illumina Hiseq2000 platform. A draft of the SfGV-ARG genome was obtained and compared to VG008. GC% content is the same for both isolates: 46.23%. Restriction analysis was performed experimentally and *in silico* in order to analyze the genome integrity and to compare it with previously published restriction patterns from Colombian and Brazilian isolates. New *Eco* RI, *Hind* III and *Bam* HI restriction sites were found in SfGV-ARG genome draft as well as the absence of some restriction sites which are present in VG008. All VG008 ORFs were found in SfGV-ARG with the exception of *lef-7*. Phylogenetic analysis was also performed in order to study SfGV-ARG relationship with the *Baculoviridae* family. SfGV-ARG was shown to belong to the same virus species described for the Colombian and Brazilian isolates although is a different variant.

Poster. **VI-32**

ssRNA viruses discovery in the fresh water snail *Biomphalaria* sp, the intermediate host of intestinal Schistosomiasis

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Viruses are present in all the living kindom from bacteria to eucaryotes. The recent contribution of Next Generation Sequencing technologies allowed increase of viral discovery in many species, including invertebrate models. In several cases viruses were demonstrated as playing key roles in host parasite interactions, like PolyDNAviruses associated with parasitoid wasps able to manipulate the immune system of their host, or like the ssRNA DcPv that modified the behavior of a ladybird parasited by a parasitoid wasp, in order to protect the parasitoid larvae. Our research focused on the immunobiological interaction between the trematode parasite *Schistosoma mansoni* and its intermediate host, the freshwater snail of the genus *Biomphalaria*. In order to better understand the interactions between the parasite and its intermediate host, and also to identify the potential presence and role of RNA viruses in this interaction, RNA sequencing was performed on several strains of *Biomphalaria* originating from different geographic areas. RNAseq data mining revealed the presence of five RNA viruses. Complete genome sequences were obtained from RNAseq transcripts by PCR joining and RACE PCR approaches. All five viruses belong to the picorna-like virus superfamily, nevertheless they differ from each others in their gene organizations and phylogenetic positions. Viral prevalences in the different strains of *Biomphalaria* were explored revealing the geographic distribution of each virus. Interestingly, if the presence of these viruses did not relies to any associated symptoms in naive snails, an increase of their transcript level has been detected for some of them following parasitic infection of *Biomphalaria glabrata* by *Schistosoma mansoni*.

Poster. **VI-33**

Studies on the role of putative replication origins from the nucleopolyhedrovirus of *Anticarsia gemmatalis* using an *in vitro* coinfection method

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Baculoviruses are arthropod-specific viruses containing dsDNA genomes of 80-180 kbp and classified in four genera: *Alpha -*, *Beta -*, *Gamma* - and *Deltabaculoviruses*. Under their interesting properties, they have been used in many applications such as biological pest control, heterologous protein expression or gene delivery in mammals. However, some mechanisms of the multiplication cycle are still poorly understood. Replication process is one of the circuits where there are confuse evidences due to the large size of baculoviral genome and the availability of methods to study, for example, what are the sequences involved in capturing the replication machinery (ORIs). Currently, transient-replication assays based on the cell transfection with recombinant plasmids containing viral sequences, the subsequent infection and finally the replicated plasmid recovery are the traditional approach to study ORIs. Numerous sequences of baculoviral genomes [e.g. homologous regions (*hr)* and other coded and non-coded regions (non-*hr)*] were associated as ORI using the mentioned methodology. While this approach is a good approximation, a major criticism is that ORIs are contained in smaller circular dsDNA than the size of a viral genome. Considering the above, in this work two putative ORIs (one classified as *hr* and the other as non-*hr)* of the nucleoplyhedrovirus of *Anticarsia gemmatalis* (AgMNPV) were introduced with *gfp* in the nucleopolyhedrovirus of *Autographa californica* (AcMNPV). Coinfection studies in UFL-Ag-286 cells (susceptible to AgMNPV and less susceptible to AcMNPV) were performed and the success of replication for recombinant AcMNPVs was measured, proposing a new approach in the characterization of baculoviral ORIs.

Poster. **VI-34**

Survey for Oryctes rhinoceros nudivirus (OrNV) in a Hawaiian coconut rhinoceros beetle (*Oryctes rhinoceros*) population and genetic diversity of Pacific isolates of OrNV

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Oryctes rhinoceros, commonly known as the coconut rhinoceros beetle (CRB) (Coleoptera: Scarabaeidae), is large invasive scarab beetle native to Southeast Asia. CRB is a major pest of coconut palm trees (*Cocos nucifera)* in its expanded range which includes tropical islands in the Pacific, and most recently the island of Oahu in the Hawaiian archipelago. For decades a double-stranded DNA virus, *Oryctes rhinoceros nudivirus* (OrNV; family *Nudiviridae*, genus *Alphanudivirus)*, has been an effective biological control agent reducing the impact of CRB in its native and expanded range. CRB arrived in Oahu in 2013 and an intensive eradication effort is currently underway. The purpose of this study is to determine whether OrNV is present in Oahu's incipient CRB population, as its presence may positively impact eradication efforts. To date, 21 field-caught CRB specimens have been assayed for OrNV using a PCR-based assay, all of which have been determined to be negative for the virus. This suggests that OrNV did not accompany CRB during its invasion of Oahu, and/or CRB on Oahu are resistant to the virus. We also assayed CRB specimens from several locations in Southeast Asia and Pacific Islands for the presence of OrNV and examined the genetic diversity of the virus within a 945 bp region of the gene coding for the gp83-like protein. OrNV could be found in all locations from which specimens were collected, with the island of Guam being an exception. Sequence analyses of OrNV isolates indicate low genetic diversity, which may limit the genotypes of CRB this virus may be able to infect.

Poster. **VI-35**

The Autographa californica multiple nucleopolyhedrovirus ac110 Gene Encodes a New *Per Os* Infectivity Factor

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The Autographa californica multiple nucleopolyhedrovirus (AcMNPV) *ac110* gene is especially conserved in lepidopteran-specific baculoviruses and is uncharacterized. To investigate the role of *ac110* in the baculovirus life cycle, an *ac110*-knockout (vAc110KO) and a repair (vAc110:HA) viruses were constructed in this study. Budded virion production and occlusion body formation were unaffected in vAc110KO-transfected or infected Sf9 cells. Intrahemocoelic injection of budded virions of vAc110KO killed *Trichoplusia ni* larvae as efficiently as the repair or the wild-type viruses. However, *per os* inoculation of occlusion bodies of vAc110KO failed to establish infection in *T. ni* larvae, while the repair virus was as efficient as the wild-type virus. Treatment with calcofluor white, a reagent that damages the peritrophic membrane, did not rescue the oral infectivity of vAc110KO. These results suggested that Ac110 is a new *per os* infectivity factor that may play a role after occlusion-derived virion passes through the peritrophic membrane during oral infection.

Poster. **VI-36**
Cancelled

Poster. **VI-37**
Cancelled

Poster. **VI-38**

The profiling of six miRNAs encoded by AcMNPV

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Besides BmNPV (Bombyx mori nucleopolyhedrovirus) and SpltMNPV (Spodoptera litura nucleopolyhedrovirus), another baculovirus, AcMNPV (Autographa californica nucleopolyhedrovirus) was also evidenced encoding miRNAs (microRNAs), and AcMNPV-miR-1 have been reported in 2013 by us. Here we present the identification and characterization of total six miRNAs encoded by AcMNPV. The candidate miRNAs were predicted through small RNA deep sequencing and bioinformatics method in AcMNPV, and then were validated be true miR- NAs by quantitative reverse transcription PCR and northern blot. Three miRNAs perfectly matched the coding sequence of three viral genes, and the latter turned into the main target logically. The other three miRNAs were located at the CDS of viral genes. The target genes were predicted both in virus and host by bioinformatics analysis and subsequently testified by dual-luciferase reporter assay. The validated targets were mainly in AcMNPV except for the targets of AcMNPV-miR-4, which were all Bombyx mori genes, thus implied that AcMNPV-miR-4 involved in viral-host interaction intensely. The function modes to targets of the six miRNAs dominant were down-regulation meanwhile minor were up-regulation. Our results suggested AcMNPV encoded miRNAs function as a pivotal modulator in insect-virus cross-talking by targeting and regulating viral own genes and (or) host genes to establish infection and replicate successfully.

Poster. **VI-39**

Virome composition of *Apis mellifera* colonies infested with *Varroa destructor*

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Varroa destructor infestation of *Apis mellifera* colonies carries and/or promotes replication of honey-bee viruses. Some of them like Deformed wing virus, Varroa destructor virus-1, Acute bee paralysis virus, Israeli acute bee paralysis virus and Kashmir virus have been well described and characterized, but others viruses associated with *Varroa* remained unknown. To learn about the viral population carried and exchanged between *V. destructor* and *Apis mellifera* in untreated Varroa-parasitized colonies we performed deep sequencing (RNA-seq), a technique that enables characterization of the virome richness, including less abundant viral components. Contig-assembly and blast analysis enabled identification of known viruses like DWV, ABPV and IAPV as well as insect and plant viruses unknown in this host-parasite system. After establishing a criteria to estimate the relevance of our findings we validated the presence of relevant new viruses in Varroa-infested honey bee colonies. Our data enabled characterization of new viruses that replicate in Varroa-infested colonies.

Poster. **VI-40**

Virus discovery and applications for the management of snail-vectored human disease

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A World Health Organization resolution targets the elimination of schistosomiasis as a public health concern by the year 2025. Taking advantage of next generation sequencing (NGS) technologies and our efficient bioinformatics pipeline developed for identification and confirmation of virus-derived sequences from NGS data, we identified virus-derived sequences from the transcriptomes of three snail vectors of schistosomiasis: *Biomphalaria glabrata, Biomphalaria pfeifferi* and *Bulinus globosus*. Sequences derived from more than 20 novel viruses were identified from 27 RNA sequence datasets, with 8 near full-length small RNA virus genomes assembled. All viral sequences showed similarity on BLAST analysis to picorna-like viruses of diatoms or unknown marine viruses. More than seven of the snail-derived virus sequences showed similarity to the dicistrovirids and iflavirids of insects, along with *Maranvirus*, *Bacillarnavirus* and *Labyrnavirus* isolated from aquatic environments. Phylogenetic analysis based on RNA-dependent RNA polymerase or coat protein sequences revealed that some virus sequences were likely derived from ingested aquatic unicellular organisms. Other virus sequences that clustered close to, but separate from insect viruses in the phylogenetic tree are hypothesized to be snail viruses. The potential practical application of such viruses for snail management will be discussed.

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