41th ANNUAL MEETING

of the

Society for

INVERTEBRATE PATHOLOGY

and

9TH INTERNATIONAL CONFERENCE ON

BACILLUS THURINGIENSIS

Incorporating COST862 Action: Bacterial Toxins for Insect Control

PROGRAM and ABSTRACTS

3-7 August 2008

University of Warwick,

Coventry, UK

SUNDAY — 3 August

8:00 – 17:00	**SIP Council Meeting**	National Grid Rm, Arts C.
10:00 – 19:00	**Registration**	Rootes Registration Desk
18:00 – 21:00	**Mixer**	Arts Centre Gallery

MONDAY — 4 August

8:00 – 18:00 **Registration** National Grid Room, Arts Centre

8:30 – 10:00 **Opening Ceremony** Arts Centre Theatre
Dr. D. Chandler, Organizing Comm.
Dr. W. Gelernter, President, SIP

Founder's Lecture
Dr. Johannes Jehle, Lecturer
André Paillot (1885-1944): His work lives on
Presentation of Founder's Lect. Award
Prof. Dudley Pinnock, Chair, Founder's Lecture Committee.
Prof. André Paillot, Honoree
Dr. Johannes Jehle, Lecturer

10:00 – 10:30 Break Arts Centre Gallery

10:30 – 12:30 **Plenary Symposium:** Arts Centre Theatre
Honey Bee Colony Collapse Disorder

12:30 – 14:00 Lunch Rootes Restaurant

12:45 – 14:00 ICTV meeting SS017

14:00 – 16:00 **Symposium:**
* Invertebrate Pathogens Arts Centre Theatre
as Models for Basic Ecological and Evolutionary Principles

14:00 – 16:00 **Contributed Papers:**
* Fungi 1 Arts Centre Conf Rm
* Microsporidia SS020
14:00 – 15:00 * Nematodes 1 SS021

16:00 – 16:30 Break Arts Centre Gallery

16:30 – 18:30 **Symposium:**
* Utilizing Insect Arts Centre Theatre
Pathogens in Green Pest Management Systems

16:30 – 18:30 **Contributed Papers:**
* Bacteria 1 SS021
* Viruses 1 Arts Centre Conf Rm

18:30 – 19:30 Dinner Rootes Restaurant

19:00 – 20:00 **Division business meetings:**
* Nematodes SS017
* Virus SS021

19:30 – 20:30 **Division business meetings:**
* Bacteria Arts Centre Conf Rm
* Fungi SS020
* Microsporidia SS009

20:00 – 21:30 **Workshop**
* Nematode Division SS017
Nematode-Bacterium Associations

20:00 – 21:30 **Workshop**
* Virus Division SS021
Invertebrate Virus Discovery

20:30 – 21:30 **Workshop**
* Fungus Division SS020
Molecular Phylogenetic Identification Resources of *Beauveria* and *Metarhizium*

20:30 – 21:30 **Workshop**
* Microsporidia Division SS009
Use of QPCR to Quantify Microsporidia Infection

TUESDAY— 5 August

6:45 – 8:00 **5K Fun Run/Walk** Warwick Univ. campus

8:00 – 10:00 **Symposium:**
* Virulence Factors in Fungal SS021
Pathogens: A Comparative Approach

8:00 – 10:00 **Contributed Papers:**
* Microbial Control 1 Arts Centre Theatre
* Nematodes 2 SS020
* Viruses 2 Arts Centre Conf Rm

10:00 – 10:30 Break Arts Centre Gallery

10:30 – 12:30 **Symposium:**
* Viruses of Bees Arts Centre Conf Rm

10:30 – 12:30 **POSTERS 1** Arts Centre Gallery
* Bacteria
* Fungi

12:30 – 14:00 Lunch Rootes Restaurant

Optional Excursion *(tickets required)*

13:30 – 18:30 **Tour** Coaches leave from Rootes bus stop (in front of Rootes Social Building). Participants will receive a packed lunch to eat on the coach.

19:00 – 23:00 **BBQ** including presentation of 5K race awards and Auction Sports Pavilion

IMPORTANT NOTE ABOUT POSTERS:
Posters should be displayed by 14:00 h on Monday in the Arts Centre Gallery.
Posters must be removed no later than 18:00 h on Thursday. Presenters should stand by their posters during the appropriate poster session.

MEALS: Meals will be served in the upstairs restaurant of the Rootes Social Building. Meals are paid for in advance. You will need to show your conference badge to restaurant staff.

CONTINUED ON INSIDE BACK COVER

WEDNESDAY— 6 August

8:00 – 10:00	**Symposium:**	
	* Entomopathogenic Bacteria Other than *Bacillus*	Arts Centre Theatre
	* Microsporidia of Aquatic Arthropods	SS020
8:00 – 10:00	**Contributed Papers:**	
	* Fungi 2	SS021
	* Viruses 3	Arts Centre Conf Rm
10:00 – 10:30	Break	Arts Centre Gallery
10:30 – 12:30	**Symposium:**	
	* Entomopathogenic Nematode Application Technology in IPM	SS020
10:30 – 12:30	**Contributed Papers:**	
	* Bacteria 2	Arts Centre Theatre
	* Microbial Control 2	SS021
	* Viruses 4	Arts Centre Conf Rm
12:00 – 14:00	**Student Workshop**	Chancellors Suite, Rootes
	***** Spreading the Word: Skills for Communicating Science and Getting it Funded	(Lunch provided)
12:30 – 14:00	Lunch	Rootes Restaurant
13:00 – 14.00	JIP Editorial Board mtg.	Chancellor 3, Rootes
14:00 – 16:00	**Symposium:**	
	* Pathogens of Bees	SS021
14:00 – 16:00	**Contributed Papers:**	
	* Bacteria 3	Arts Centre Theatre
	* Nematodes 3	SS020
	* Viruses 5	Arts Centre Conf Rm
16:00 – 16:30	Break	Arts Centre Gallery
16:30 – 18:30	**POSTERS 2**	Arts Centre Gallery
	* Microbial Control	
	* Microsporidia	
	* Nematodes	
	* Other	
	* Viruses	
17:00 – 18:30	COST meeting	SS020
18:30 – 19:30	Dinner	Rootes Restaurant
18:15 – 18:45	Buffet for MC business mtg participants	Arts Centre Ensemble Rm
18:45 – 19:30	**Division business meeting:**	
	* Microbial Control	Arts Centre Conference Rm
19:30 – 21:30	**Workshop**	
	* Microbial Control Biological Solutions to Pest Control	Arts Centre Conference Rm
21:30	* Mixer	Arts Centre Theatre Bar

THURSDAY— 7 August

8:00 – 10:00	**Symposia:**	
	* Commercialization and Quality Control of Bacterial Insecticides	Arts Centre Theatre
	* Comparative Genomics of DNA Viruses	Arts Centre Conf Rm
8:00 – 10:00	**Contributed Papers:**	
	* Pathogens of Bees	SS021
10:00 – 10:30	Break	Arts Centre Gallery
10:30 – 12:30	**SIP ANNUAL BUSINESS MEETING**	Arts Centre Theatre
12:30 – 14:00	Lunch	Rootes Restaurant
13:00 – 14:00	Student Committee meeting	SS020
12:45 – 14:00	Student Awards Committee meeting	SS017
14:00 – 16:00	**Symposium:**	
	* Role of Disease in Regulation of Non-Pest Populations	Arts Centre Theatre
14:00 – 16:00	**Contributed Papers:**	
	* Bacteria 4	Arts Centre Conf Rm
	* Microbial Control 3	SS021
16:00 – 16:30	Break	Arts Centre Gallery
16:30 – 18:30	**Symposium:**	
	* Regulatory and Market Barriers for Approval of Microbial Control Products	Arts Centre Theatre
16:30 – 18:30	**Contributed Papers:**	
	* Bacteria 5	SS021
	* Viruses 6	Arts Centre Conf Rm
19:00	Coaches leave from Rootes bus stop (in front of Rootes Social Bldg.)	
19:00	**BANQUET AND AWARDS CEREMONY**	Britannia Royal Court Hotel
19:00 – 20:00	Cocktail Hour	
20:00	Banquet	
22:30 – 00:30	Coaches return to campus	
00:30 on	Return by taxi	

KEY TO MEETING ROOMS

SS – Social Studies
Arts Centre Conf Rm – Arts Centre Conference Room
Rootes – Rootes Social Building

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Society for Invertebrate Pathology

President	**Wendy Gelernter**	Pace Consulting, 1267 Diamond St, San Diego, CA 92109, USA Phone / Fax: (858) 272-9897 / (858) 483-6349 Email: gelernt@paceturf.org
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Yasuhisa Kunimi

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Andreas Linde (Chair)
Bryony Bonning
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Co-Chair:	Doreen Winstanley
Program:	Bryony Bonning
Local Arrangements and Conference Coordinator:	Heike Kuhlmann

Please join the Organizing Committee and SIP in gratefully acknowledging the invaluable contributions and efforts of the following:

5K Race and COST co-ordinator	Neil Crickmore
Miscellaneous assistance	Robert Possee, Judith Pell, Helen Roy Gill Prince, Trish Wells, Gary Keane, Sally Hilton John Danquah, David Carpentier, Zenas George Vidisha Krishnan, Paul Johnston, Nick Jessop

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PROGRAM

2008

IMPORTANT NOTES:

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

STU indicates papers being judged for graduate student presentation awards

129 indicates abstract number for ORAL presentation

B-11 indicates abstract number for POSTER presentation

SUNDAY - 3 August

8:00–17:00	SIP Council Meeting	**National Grid Rm, Arts Cntr.**
10:00–19:00	Registration	**Rootes Conference Desk**
18:00–21:00	Mixer	**Arts Centre Gallery**

MONDAY - 4 August

8:00–18:00 Registration **National Grid Room, Arts Centre**

Monday, 8:30-10:00. Arts Centre Theatre

Opening Ceremonies and SIP Founders' Memorial Lecture

Opening Ceremonies

David Chandler, Chair, Organizing Committee
Wendy Gelernter, President, SIP

Founders' Memorial Lecture

Dudley Pinnock, Chair, Founders' Lecture Committee
Honoree: **ANDRÉ PAILLOT**
Lecturer: **JOHANNES JEHLE**

André Paillot (1885-1944): His work lives on

10:00–10:30 **BREAK** Arts Centre Gallery

Plenary Symposium Monday, 10:30–12:30. Arts Centre Theatre

Honey Bee Colony Collapse Disorder

Organizers: Diana Cox-Foster and Bryony Bonning.
Moderator: Diana Cox-Foster.

10:30 **1** **Colony Collapse Disorder (CCD): CSI in the bee hive** Dennis vanEngelsdorp[1], [1]Pennsylvania State Department of Agriculture, Harrisburg, Pennsylvania, USA

11:00 **2** **Microsporidia infections in hymenopteran pollinators** Ingemar Fries[1], [1]Department of Entomology, Swedish University of Agricultural Sciences, Uppsala, Sweden

11:30 **3** **Applied beenomics: Molecular studies of honey bee disease and resistance** Jay D. Evans[1], [1]USDA, ARS, Beltsville, MD, USA

12:00 **4** **Unraveling the pathogens in honey bees undergoing Colony Collapse Disorder** Diana Cox-Foster, Dept of Entomology, Penn State University, University Park, PA, USA

12:30–14:00 **LUNCH** Rootes Restaurant

Symposium (Cross Divisional) Monday, 14:00-16:00. Arts C. Theatre

Invertebrate Pathogens as Models for Basic Ecological and Evolutionary Principles

Organizer/Moderator: Elizabeth Davidson.

14:00 **5** **Where theory meets reality: Viral disease in field populations of forest Lepidoptera** Jenny Cory[1]; Judy Myers[2], [1]Algoma University College , Sault Ste. Marie, Ontario Canada and Simon Fraser University, Burnaby, BC, Canada; [2]University of British Columbia, Vancouver, BC, Canada

14:30 **6** **Baculoviruses as a model of host shifts and disease emergence** Amy B. Pedersen[1], [1]University of Sheffield, UK

15:00 **7** **Host-parasite coevolution under environmental variation** Tom J. Little[1], [1]University of Edinburgh, UK

15:30 **8** **The evolutionary ecology of Bt** Michael B. Bonsall[1], [1]Oxford University, UK

Contributed Papers Monday, 14:00-15:45. Arts C. Conf Rm.

FUNGI 1

Moderator: Everton Fernandes.

14:00 **9** **The fascinating true story about the famous *Metarhizium anisopliae* isolate Ma43, alias ATCC 90448, alias BIPESCO 5, alias F52 alias ……..** Jørgen Eilenberg[1]; Gisbert Zimmermann[2]; Tariq Butt[3]; Kerstin Jung [2]; Charlotte Nielsen[1]; Hermann Strasser[4]; Milton Typas[5]. [1]University of Copenhagen, Denmark; [2]BBA, Institute for Biological Control, Darmstadt, Germany; [3]University of Swansea, Wales, UK; [4]University of Innsbruck, Austria; [5]University of Athens, Greece

14:15 **10** **A novel approach to develop biopesticides based on entomopathogenic fungi** Kim . Jae Su[1]; Woo Eun Ok[1]; Park Jong Sung[1]; Kim Yun Sung[1]; Kim Tae-Joon[1]; Kim Kyoung-Sung[1]; Roh Jong Yul[2]; Choi Jae Young[2]; Je Yeon Ho[2],. [1]AgroLife Research Institute, Dongbu HiTek Co. Ltd., Korea; [2]Seoul National University, Korea

14:30 **11 STU** **Host plant effects on fitness of the mite pathogenic fungus *Neozygites floridana*** Vitalis W. Wekesa[1]; Stefania Vital[1]; Renan A. Silva[1]; Italo Delalibera Jr.[1], [1]University of São Paulo, Brazil

14:45 **12** **Intraguild interactions involving *Pandora neoaphidis* at the population scale** Jason Baverstock[1]; Judith K. Pell[1], [1]Rothamsted Research, Harpenden, Hertfordshire, UK

15:00 **13 STU** **Enhanced transmission of *Pandora neoaphidis* by the invasive ladybird *Harmonia axyridis*** Patricia M. Wells[1]; Jason Baverstock[1]; Michael E.N. Majerus[2]; Helen E. Roy[3]; Judith K. Pell[1], [1]Rothamsted Research, Harpenden, Hertfordshire, UK; [2]University of Cambridge, UK; [3]Centre for Ecology and Hydrology, Huntingdon, Cambridgeshire, UK

15:15 **14** **Liquid media carbon/nitrogen ratio affects the insecticidal activity of the crude soluble protein extract of *Metarhizium anisopliae* 01/58-Su strain against medfly *Ceratitis capitata* (Diptera; Tephritidae) adults** Almudena Ortiz-Urquiza[1]; Ana Borrego[1]; Cándido Santiago-Álvarez[1]; Enrique Quesada-Moraga[1], [1]University of Córdoba, Spain

15:30 **15** **Viability of formulations of *Beauveria bassiana* for use in grain stores** Bryony Taylor[1]; Belinda Luke[1], [1]CABI, Bakeham Lane, Egham, Surrey, UK

Contributed Papers Monday, 14:00-16:00. SS020

MICROSPORIDIA

Moderator: Lee Solter.

14:00 **16** **Microsporidian pathogens of the oak processionary moth, *Thaumetopoea processionea* (Lep., Notodontidae), and their potential for inoculative release** Gernot Hoch[1]; Axel Schopf[1], [1]BOKU University of Natural Resources and Applied Life Sciences Vienna, Austria

14:15 **17** **Effects of a microsporidium from the convergent lady beetle *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae) on three non-target coccinellids** Taro Saito[1]; Susan Bjornson[1], [1]Saint Mary's University, Halifax, Canada

14:30 **18 STU** **Ultrastructure and pathology of a microsporidium from the convergent lady beetle, *Hippodamia convergens* Guerin-Meneville** Jeffrey Le[1]; Susan Bjornson[1], [1]Saint Mary's University, Halifax, Canada

14:45 **19 STU** **Life cycle of a microsporidian isolate (*Nosema* sp.) from the three spot grass yellow butterfly, *Eurema blanda arsakia*** Yi-chun Tsai[1], Chung-Hsiung Wang[1], [1]National Taiwan University, Taiwan (R.O.C)

15:00 **20 STU** **A new species, *Vairimorpha ocinarae* n. sp., isolated from *Ocinara lida* Moore (Lepidoptera: Bombycidae) in Taiwan** Chih-Yuan Wang[1]; Wei-Fong Huang[1]; Yi-Chun Tsai[1]; Chung-Hsiung Wang[1], [1]National Taiwan University, Taiwan (R.O.C)

15:15 **21** **A new microsporidian species isolated from the freshwater shrimp, *Caridina formosae*** Tai-Chuan Wang[1]; Chih-Yuan Wang[1]; Wei-Fone Huang[1]; Chung-Hsiung Wang[1], [1]National Taiwan University, Taiwan (R.O.C)

15:30 **22** **Rapid DNA extraction from microsporidian spores of insect origin** Wei-Fone Huang[1]; Leellen Solter[2]; Chih-Yuan Wang[1]; Yi-Ting Yang[1]; Chung-Hsiung Wang[1], [1]National Taiwan University, Taiwan; [2]Illinois Natural History Survey, Illinois, USA

15:45 **23** **Prevalence rates and genetic diversity of microsporidia associated with European corn borer *Ostrinia spp.* (Lepidoptera: Crambidae) in France** Yuri S. Tokarev[1]; Julia M. Malysh[1]; Philippe Audiot[2]; Igor V. Senderskiy[1]; Andrei N. Frolov[1]; Sergine Ponsard[3]; Denis Bourguet[3], [1]All-Russian Institute for Plant Protection RAAS, Russia; [2]Centre de Biologie et de Gestion des Populations, Montferrier-sur-Lez, France; [3]Université Paul Sabatier - Toulouse III, France

Contributed Papers — Monday, 14:00-15:00. SS021

NEMATODES 1

Moderators: Patricia Stock and Lorena Uribe-Lorio.

14:00 **24** **Development and ultrastructure of the bacterial receptacle in *Steinernema* nematodes (Nematoda: Steinernematidae)** S.Patricia Stock[1]; Sam K. Kim[1]; Yolanda Flores-Lara[1], [1]University of Arizona, Tucson AZ USA

14:15 **25** **Biochemical and molecular characterization of symbiotic bacteria of four *Steinernema* from Costa Rica, *S. costaricense* n.sp.(CR9), *S. puntauvense* n. sp. (Li6), *S. websterii* (CR5) and *Steinernema sp.* (T4)** Lorena Uribe-Lorío[1]; S. Patricia Stock[2]; Diego Navarro[1]; Elena Castillo[1]; Marielos Mora[1], [1]University of Costa Rica, Costa Rica; [2]University of Arizona, Tucson, AZ, USA

14:30 **26** ***Bacillus* bacteria and their fitness consequences on *Pristionchus* nematodes** Robbie Rae[1]; Ralf J. Sommer[1], [1]Max Planck Institute for Developmental Biology, Germany

14:45 **27** **Suppressive effects of metabolites from *Photorhabdus* spp. and *Xenorhabdus* spp. on phytopathogens of peach and pecan** David Shapiro-Ilan, Charles C. Riley, Michael W. Hotchkiss; USDA-ARS, Byron, GA, USA

16:00–16:30 **BREAK** Arts Centre Gallery

Symposium (Div. of Microbial Control) — Monday, 16:30-18:30. Arts C. Theatre

Utilizing Insect Pathogens in Green Pest Management Systems

Organizers/Moderators: Dawn Gouge, Michael Wilson, Michael Brownbridge.

16:30 **28** **The long and winding road – discovery to commercial product: Are we there yet?** Michael Brownbridge[1], [1]AgResearch Ltd., New Zealand

16:50 **29** **Exploring tritrophic interactions: Biological control of an obligate pest by its obligate parasite** Keith G. Davies[1], [1]Rothamsted Research, Harpenden, Hertfordshire UK

17:10 **30** **Proposals for improved registration requirements for microbial biological control agents** Ralf-Udo Ehlers[1], [1]Christian-Albrechts-University Kiel, Germany

17:30 **31** **Use of microbial agents in urban pest management systems** Dawn H. Gouge[1], [1]University of Arizona, USA

17:50 **32** **Conservation biological control strategies with entomopathogenic fungi: Potential and perspectives** Judith K. Pell[1], Rothamsted Research, Harpenden, Hertfordshire, UK

18:10 **33** **Entomopathogenic nematodes market diversity** Peters Arne[1], [1]e-nema, Germany

Contributed Papers — Monday, 16:30-18:30. SS021

BACTERIA 1

Moderator: Colin Berry.

16:30 **34** **Structural and mutational analysis of the receptor-binding domain of Cry4Aa mosquito-larvicidal protein** Panadda Boonserm[1]; Min Mo[2]; Chanikarn Boonchoy[3]; Julien Lescar[2], [1]Mahidol University, Thailand; [2]Nanyang Technological University, Singapore; [3]Mahidol University, Thailand

16:45 **35 STU** **Effect of the *Bacillus thuringiensis* Cry4Ba toxin on the peritrophic membrane in *Aedes aegypti* mosquito larvae** Seangdeun Moonsom[1]; Urai Chaisri[1]; Ping Wang[2]; Chanan Angsuthanasombat[1], [1]Mahidol University, Thailand; [2]New York State Agricultural Experiment Station, Cornell University, Geneva, NY USA

17:00 **36 STU** **Inter-molecular interaction between *Bacillus thuringiensis* Cry4Ba and Cry4Aa mosquito-larvicidal proteins in lipid membranes results in enhanced toxicity** Narumol Khomkhum[1]; Boonhiang Promdonkoy[2]; Chanan Angsuthanasombat[1]; Panadda Boonserm[1], [1]Mahidol University, Thailand; [2]National Science and Technology Development Agency, Thailand

17:15 **37 STU** **Functional analysis of the truncated BinA component of the binary toxin from *Bacillus sphaericus*** Suweeraya Limpanawat[1]; Panadda Boonserm[1]; Boonhiang Promdonkoy[2], [1] Mahidol University, Thailand; [2]National Science and Technology Development Agency, Thailand

17:30 **38 STU** **Amino acid substitutions in selected regions of *Bacillus sphaericus* BinB toxin revealed residues important for toxicity** Kamonnut Singkhamanan[1]; Panadda Boonserm[1]; Boonhaing Promdonkoy[2], [1]Mahidol University, Thailand; [2]National Science and Technology Development Agency, Thailand

17:45 **39 STU** **Loop2 in Cry4Aa domain II, but not loops 1 and 3, is essential for the mosquitocidal activity against *Culex pipiens*** Mohammad Tofazzal Hossain Howlader[1]; Yasuhiro Kagawa[1]; Hiroshi Sakai[1]; Tohru Hayakawa[1], [1]Okayama University, Okayama, Japan

18:00 **40 STU** **Identification of the midgut binding-molecule for Cry4Ba toxin in *Anopheles albimanus* larvae** Maria Teresa Fernandez-Luna[1]; Alejandra Bravo[1]; Humberto Lanz[2]; Sarjeet Gill[3]; Mario Soberon[1]; Juan Miranda-Rios[1], [1]National Autonomous University of Mexico, Morelos, Mexico; [2]Instituto Nacional de Salud Publica, Morelos, Mexico; [3]University of California, Riverside, USA

18:15 **41 STU** **Novel insecticidal crystal protein genes of *Bacillus thuringiensis* strains isolated from soil samples in China** Meng Ying[1]; Song Rong[1]; Zhang Zhenyu[1]; Zhu Zimin[1]; Sun Ming[1]; Yu Ziniu[1], [1]Huazhong Agricultural University, Wuhan, P. R. China

Contributed Papers Monday, 16:30-18:30. Arts C. Conf. Rm.

VIRUSES 1

Moderators: Robert Possee and Zhihong Hu.

16:30 **42** **Phylogenetic approaches to delimit baculovirus species based on single gene and whole genome data** Elisabeth A. Herniou[1]; Jennifer S. Cory[2]; Timothy G. Barraclough[1], [1]Imperial College London, Ascot, Berskshire, UK; [2]Algoma University College, Sault Ste. Marie, Ontario, Canada

16:45 **43** **Genome sequence of the complete genotype of *Spodoptera frugiperda* multiple nucleopolyhedrovirus isolate from Nicaragua** Oihane Simón[1]; Delia Muñoz[1]; Trevor Williams[2]; Primitivo Caballero[1]; Miguel López-Ferber[3], [1]Universidad Pública de Navarra, Navarra, Spain; [2]Instituto de Ecología AC, Veracruz, Mexico; [3]Ecole des Mines d'Alès, Alès, France

17:00 **44 STU** **Genomic and host range study of the smallest lepidopteran NPV, *Maruca vitrata* multiple nucleopolyhedrovirus** Yun-Ru Chen[1]; Chih-Yu Wu[1]; Song-Tay Lee[2]; Yan-Jheng Wu[2]; Meng-Feng Tsai[3]; Chu-Fang Lo[1]; Chung-Hsiung Wang[1], [1]National Taiwan University, Taipei, Taiwan, R.O.C; [2]Southern Taiwan University of Technology, Tainan, Taiwan, R.O.C.; [3]Dayeh University, Changhua, Taiwan, R.O.C.

17:15 **45 STU** **Comparative genomics of different isolates of *Cydia pomonella* granulovirus (CpGV)** Karolin E. Eberle[1]; Doreen Winstanley[2]; Mohammadreza Rezapanah[3]; Johannes A. Jehle[1], [1]Agricultural Service Center Palatinate (DLR), Germany; [2]Warwick Horticulture Research International, University of Warwick, Wellesbourne, UK; [3]Insect Biocontrol Research Department, Tehran, Iran

17:30 **46 STU** **A new nucleopolyhedrovirus of *Lymantria xylina* Swinhoe (Lepidoptera: Lymantriidae) with a defective fp25 gene from Taiwan** Yu-Shin Nai[1]; Tai-Chuan Wang[1]; Yun-Ru Chen[1]; Chung-Hsiung Wang[1], [1]National Taiwan University, Taipei, Taiwan (R.O.C)

17:45 **47** **Sequence and organization of the *Orgyia leucostigma* nucleopolyhedrovirus genome** Renée Lapointe[1]; Robert J.M. Eveleigh[1]; Robert I. Graham[2]; Hillary A.M. Lauzon[3]; Lilian Pavlik[3]; Basil M. Arif[3]; Christopher J. Lucarotti[4], [1]Sylvar Technologies Inc., Fredericton, New Brunswick, Canada; [2]CSIRO Entomology, Canberra, Australia; [3]Natural Resources Canada, Canadian Forest Service – Great Lakes Forestry Centre, Ontario, Canada; [4]Natural Resources Canada, Canadian Forest Service - Atlantic Forestry Centre, New Brunswick, Canada

18:00 **48** **Comparative analysis of the genome sequence of two isolates of *Glossina pallidipes* salivary gland hypertrophy virus (GpSGHV) from Uganda and Ethiopia** Adly M.M. Abd-Alla[1]; François Cousserans[2]; Andrew Parker[1]; Alan Robinson[1]; Max Bergoin[2], [1]International Atomic Energy Agency, Vienna, Austria; [2]Université Montpellier II, France

18:15 **49** **Comparative analysis of viruses that cause salivary gland hypertrophy in *Glossina pallidipes* (GpSGHV) and *Musca domestica* (MdSGHV)** Alejandra Garcia-Maruniak[1]; Adly M. M. Abd-Alla[2]; Andrew G. Parker[2]; Tamer Z. Salem[1]; Verena-Ulrike Lietze[1]; Monique M. van Oers[3]; James E. Maruniak[1]; François Cousserans[4]; Alan S. Robinson[2]; Just M. Vlak[3]; Max Bergoin[4]; Drion G. Boucias[1], [1]University of Florida, Gainesville, FL USA; [2]FAO/IAEA Seibersdorf, Austria; [3]Wageningen University, The Netherlands; [4]Université Montpellier II, France

DINNER
18:30–19:30 Rootes Restaurant

SIP Division Business Meetings: Monday evening

Nematode	(19:00-20:00) SS017
Viruses	(19:00-20:00) SS021
Bacteria	(19:30-20:30) Arts Centre Conf. Rm.
Fungi	(19:30-20:30) SS020
Microsporidia	(19:30-20:30) SS009

Nematode Division Workshop Monday, 20:00-21:30. SS017

Nematode-Bacterium Associations

Organizer: Patricia Stock.

20:00 **50** **Nematode-Bacteria Symbiosis Research Network: Intertwining knowledge and research tools** S.Patricia Stock[1], [1]University of Arizona, USA

20:15 **51** **Evolution and genetics of *C. elegans*-pathogen interactions** Hinrich Schulenburg[1], [1]Westphalian Wilhelms-University, Germany

20:30 **52** **Innate immunity in nematodes and somaclonal cuticle variation as revealed by *Pasteuria penetrans*** Keith G. Davies[1], [1]Rothamsted Research, Harpenden, Hertfordshire, UK

20:45 **53** **The obligate *Wolbachia* endosymbiont in filarial nematodes provides potential targets for disease intervention** Barton E. Slatko[1]; Bo Wu[1]; Jeremy Foster[1], [1]New England Biolabs, Inc., Ipswich MAUSA

21:00 **54** ***Photorhabdus:* Molecular analyses of pathogenicity and mutualism** Catherine A. Easom[1]; David J. Clarke[1], [1]University College Cork, Ireland

Virus Division Workshop Monday, 20:00-21:30. SS021

Invertebrate Virus Discovery

Organizers: Peter Krell and James Maruniak.

20:00 **55** **Hunting for insect pathogens: A genomics approach** Wayne B. Hunter[1] [1]USDA, ARS, Fort Pierce, FLUSA

20:30 **56** **Discovering nucleopolyhedrovirus and iridescent viruses of *Spodoptera* spp.** Trevor Williams[1]; Oihane Simón[2]; Gabriel Clavijo[2]; Delia Muñoz[2]; Rosa Murillo[2]; Primitivo Caballero[2]; Robert D. Possee[3]; Noe Hernández[4]; Jorge E. Ibarra[4]; Miguel López-Ferber[5], [1]Instituto de Ecología AC, Xalapa, Mexico; [2]Universidad Pública de Navarra, Pamplona, Spain; [3]CEH-Oxford, UK; [4]CINVESTAV-IPN, Mexico; [5]LGEI, Ecole des Mines d'Alés, Alés, France

Fungus Division Workshop Monday, 20:30-21:30. SS020

Molecular Phylogenetic Identification Resources for *Beauveria* and *Metarhizium*

Organizers: Stephen Rehner, Joseph Bischoff, Richard Humber.

20:30 **57** **Web-based molecular phylogenetic identification resources for *Beauveria* and *Metarhizium*** Stephen A. Rehner[1]; Joseph F. Bischoff[2]; Richard A. Humber[3], [1] USDA-ARS, Beltsville, MD USA; [2]USDA-APHIS, Beltsville, MD USA; [3]USDA-ARS, Ithaca, NY, USA

Miocrosporidia Division Workshop Monday, 20:30-21:30. SS009

Use of QPCR to Quantify Microsporidia Infection

Organizer: David Oi.

20:30 **58** **Quantifying developing *Thelohania solenopsae* infections in the red imported fire ant, *Solenopsis invicta*** Steven Valles[1]; David Oi[1], [1]USDA-ARS, Gainesville, FL, USA

21:00 **59** **Prevalence and levels of *Nosema ceranae* in healthy and declining honey bee colonies** Yanping Chen[1]; Jay D. Evans[1], [1]USDA-ARS Bee Research Lab, Beltsville, MD, USA

TUESDAY - 5 August

6:45 5K Fun Run / Walk Warwick Univ Campus

Symposium (Div. of Fungi) Tuesday, 8:00-10:00. SS021

Virulence Factors in Fungal Pathogens: A Comparative Approach

Organizer/Moderator: Enrique Quesada-Moraga.

8:00 **60** **Pathogenicity determinants of the entomopathogenic fungi *Metarhizium anisopliae*** Raymond J. St. Leger[1]; Chengshu Wang[2]; Weiguo Fang[1]; Sibhao Wang[1],[1]University of Maryland, USA; [2]Shanghai Institutes for Biological Sciences, PRC

8:24 **61** **Attenuation of virulence in entomogenous fungi** Tariq M. Butt[1]; Farooq A. Shah[1], [1]Swansea University, UK

8:48 **62** **Developing insect models to study the evolution of fungal pathogens** Michael J. Bidochka[1], [1]Brock University, Canada

9:12 **63** **Investigating the biology of plant infection by the rice blast fungus *Magnaporthe grisea*** Martin J. Egan[1]; Michael J. Kershaw[1]; Diane O. Saunders[1]; Elise Lambeth[1]; Ana-lilia Martinez-Rocha[2]; Nicholas J. Talbot[1], [1]University of Exeter, UK; [2]Universidad de Córdoba, Córdoba, Spain

9:36 **64** **Are there overlaps between virulence factors of fungal pathogens of arthropods, plants, and vertebrates?** Alice C.L. Churchill[1], [1]Cornell University, Ithaca, NY, USA

Contributed Papers Tuesday, 8:00-9:45. Arts C. Theatre

MICROBIAL CONTROL 1

Moderators: Trevor Jackson and Barbara Manachini.

8:00 **65** **Bioinsecticide based on *Bacillus thuringiensis* subsp. *kurstaki* delta-endotoxins for the control of the lepidopteran olive tree pathogenic insect *Prays oleae*: From gene cloning to application in the field** Samir Jaoua[1]; Souad Rouis[1]; Slim Tounsi[1]; Nabil Zouari[1]; Imène Saadaoui[1]; Hichem Azzouz [1]; Lobna Abdelkafi Mesrati[1], [1]Centre of Biotechnology of Sfax., Tunisia

8:15 **65a** **The influence of *Bacillus thuringiensis* on baculovirus transmission dynamics in the cabbage moth, *Mamestra brassicae*** Helen Hesketh[1]; Rosemary S. Hails[1], [1]NERC Centre for Ecology and Hydrology, Oxford, UK

8:30 **66 STU** **Effectiveness of *Bt* chickpeas and the entomopathogenic fungus *Metarhizium anisopliae* to control *Helicoverpa armigera* (Lepidoptera: Noctuidae)** Nora C. Lawo[1]; Rod J. Mahon[2]; Richard J. Milner[2]; Bidyut K. Sarmah[3]; Thomas J.V. Higgins[4]; Jörg Romeis[1], [1]Agroscope Reckenholz-Tänikon Research Station ART, Zurich, Switzerland; [2]CSIRO Entomology, Canberra, Australia; [3]Assam Agricultural University, Jorhat India; [4]CSIRO Plant Industry, Canberra, Australia

8:45 **67 STU** **The role of population structure in determining *Bacillus thuringiensis* resistance in cabbage loopers, *Trichoplusia ni*** Michelle T. Franklin[1]; Judith H. Myers[1], [1]University of British Columbia, Vancouver, BC, Canada

9:00 **68** **Effects of *Diabrotica*-resistant Cry3Bb1-Bt-maize on saprophagous Diptera and their coleopteran predators** Wolfgang Buechs[1]; Oliver Schlein[1]; Sabine Prescher[1], [1]Federal Research Centre for Cultivated Plants, Germany

9:15 **69** **Preliminary results on the interaction between *Bacillus thuringensis* and Red Palm Weevil** Barbara Manachini[1]; Valentina Mansueto[1]; Vincenzo Arizza[1]; Nicolò Parrinello[1], [1]University of Palermo, Italy

9:30 **70** **Genomics of the silkworm *Bombyx mori*: Tissue specificity and time course of gene expression in response to parasitization by tachinid flies** Andrew Kalyebi[1]; Y. Nakamura[1]; K. Mita[1]; H. Noda[1]; R. Ichiki[2]; S. Nakamura[2]; K. Kadono-Okuda[1],[1]National Institute of Agrobiological Sciences, Tsukuba, Japan; [2]Japan International Research Center for Agricultural Sciences, Tsukuba, Japan

Contributed Papers Tuesday, 8:00-9:45. SS020

NEMATODES 2

Moderators: Albrecht Koppenhöfer and Dawn Gouge.

8:00 **71** **Unravelling interspecific variability in virulence of four entomopathogenic nematodes to four white grub species: Virulence, infectivity, penetration sites** Albrecht M. Koppenhöfer[1]; Eugene M. Fuzy[1], [1]Rutgers University, New Brunswick, NJ, USA

8:15 **72 STU** **Diversity of nematodes parasitizing slugs in the United States of America and the United Kingdom** Jenna Ross[1]; Sergei Spiridonov[2]; Elena Ivanova[2]; Jeremy Pearce[3]; Paul Severns[4]; Graeme Nicol[1]; Michael Wilson[1], [1]University of Aberdeen, UK; [2]Russian Academy of Sciences, Moscow, Russia; [3]Becker Underwood, Littlehampton, West Sussex, UK; [4]Oregon State University, Corvallis, Oregon, USA

8:30 **73 STU** **Can endemic entomopathogenic nematode populations be used in conservation biological control of the annual bluegrass weevil (*Listronotus maculicollis*)?** Benjamin A. McGraw and Albrecht M. Koppenhöfer, Rutgers University, New Brunswick, NJ, USA

8:45 **74 STU** **Development of a controlled release system for EPN application** Melita Zec-Vojinovic[1]; Heikki M.T. Hokkanen[1], [1]University of Helsinki, Finland

9:00 **75** **Formulation and application of entomopathogenic nematode infected cadavers for control of *Hoplia philanthus* in turf** Hussain Abid[1]; Ansari A. Minshad[2]; Moens Maurice[3], [1]Sardar Vallabh Bhai Patel University of Agriculture & Technology, India; [2] Swansea University, UK; [3]Institute for Agriculture and Fisheries Research, Merelbeke, Belgium

9:15 **76** **Managing chickpea pod borer, *Helicoverpa armigera* (Hübner) with *Heterorhabitis indica*: A success story** Prabhuraj Aralimarad[1]; B V. Patil[1]; K S. Girish[1]; Shivaleela Shivaleela[1], [1]University of Agricultural Sciences, Dharwad, Raichur, India

9:30 **77** **Potential for biocontrol of *Diaprepes abbreviatus* larvae in nurseries in southern California** Kenneth O. Spence[1]; Edwin E. Lewis[1]; Jim Bethke[2], [1]University of California-Davis, USA; [2]UCCE-San Diego County, San Marcos, CA, USA

Contributed Papers Tuesday, 8:00-10:00. Arts C. Conf. Rm.

VIRUSES 2

Moderators: Linda King and Miguel Lopez-Ferber.

8:00 **78** **Functional studies of *per os* infectivity factors of *Helicoverpa armigera* single nucleopolyhedrovirus** Jingjiao Song[1]; Ranran Wang[1]; Fei Deng[1]; Hualin Wang[1]; Zhihong Hu[1], [1]Wuhan Institute of Virology, Chinese Academy of Sciences, P. R. China

8:15 **79 STU** **Influence of *pif* and *pif*2 genes in the dynamics of recombinant insect virus populations** Gabriel Clavijo[1]; Oihane Simon[1]; Delia Muñoz[1]; Martine Ceruti[2]; Trevor Williams[3]; Primitivo Caballero[1]; Miguel Lopez-Ferber[4], [1]Universidad Publica de Navarra, Spain; [2]CNRS, Saint Christol-Les-Alés, France; [3]Instituto de Ecología AC, Xalapa, Veracruz, Mexico; [4]Ecole des Mines d'Alès, Alès, France

8:30 **80** ***Ac*MNPV *ac143* (*odv-e18*), a core gene that forms a cluster with *ac142*, is essential and mediates BV production** Christina B. McCarthy[1]; Cam Donly[2]; David A. Theilmann[1], [1]Agriculture and Agri-Food, Summerland, BC, Canada; [2]Agriculture and Agri-Food Canada, London, Ont., Canada

8:45 **81 STU** **38K is a novel baculovirus nucleocapsid protein that interacts with other nucleocapsid proteins (VP1054, VP39 and VP80) and itself in *Autographa californica* multiple nucleopolyhedrovirus** Wenbi Wu[1]; Hanquan Liang[1]; Chao Liu[1]; Meijing Yuan[1]; Kai Yang[1]; Yi Pang[1], [1]Sun Yat-sen University, Guangzhou, China

9:00 **82** **The transmembrane domain of the AcMNPV GP64 protein plays specific roles in membrane fusion and virion budding** Zhaofei Li[1]; Gary W. Blissard[1], [1]Boyce Thompson Institute at Cornell University, Ithaca, NY, USA

9:15 **83 STU** **The F-like protein of group I NPVs enhances the production and infectivity of the budded virus of *gp64*-null AcMNPV pseudotyped with the envelope fusion protein F of group II NPVs** Manli Wang[1]; Ying Tan[1]; Feifei Yin[1]; Fei Deng[1]; Zhihong Hu[1]; Just M. Vlak[2]; Hualin Wang[1], [1]Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, China; [2]Wageningen University, The Netherlands

9:30 **84 STU** **A highly conserved baculovirus gene *p48* is essential for BV production and ODV envelopment** Meijin Yuan[1]; Wenbi Wu[1]; Chao Liu[1]; Yanjie Wang[1]; Chaoyang Hu[1]; Kai Yang[1]; Yi Pang[1], [1]Sun Yat-sen University, Guangzhou, China

9:45 **85 STU** **The baculovirus P10 protein and cellular microtubules are involved in the final stage of polyhedron formation** David C. J. Carpentier[1]; Caroline M. Griffiths[1]; Linda A. King[1], [1]Oxford Brookes University, Oxford, UK

10:00–10:30 **BREAK** Arts Centre Gallery

Symposium (Div. of Viruses) Tues., 10:30-12:30. Arts C. Conf. Rm.

Viruses of Bees

Organizer/Moderator: Diana Cox-Foster

10:30 **86** **What could be the association of IAPV and CCD and protecting bees from IAPV** Ilan Sela[1,2]; Eyal Maori[1]; Nitzan Paldi[2]; Eitan Glick[2], [1]The Hebrew University of Jerusalem, Israel; [2]Beeologics, LLC, Miami, FL, USA

11:00 **87** **The pitfalls of diagnosis interpretation in honey bee pathology: The case of deformed wing virus (DWV)** Laurent Gauthier[1]; Julie Fievet[1]; Diana Tentcheva[1]; Marc Edouard Colin[1]; Max Bergoin[2], [1]SupAgro Montpellier, Montpellier, France; [2]Université Montpellier 2, Montpellier, France

11:30 **88** **Transmission and pathogenesis of DWV** Sebastian Gisder[1]; Constanze Yue[1]; Elke Genersch[1], [1]Institute for Bee Research, Germany

12:00 **89** **Host specificity of honey bee viruses and transmission routes—Implications for pollinator health** Rajwinder Singh[1], Abby Kalkstein[1], Edwin Rajotte[1], Dennis vanEngelsdorp[3], Nancy Ostiguy[1], Eddie Holmes[2], Claude dePamphilis[2], Rick Donvall[3], Ian Lipkin[4], Diana Cox-Foster[1], [1]Dept of Entomology, and [2]Dept of Biology, Penn State University, University Park, PA, USA; [3]Pennsylvania Dept of Agriculture, Harrisburg, PA, USA; [4]Mailman School of Public Health, Columbia University, USA

Tuesday, 10:30-12:30. Arts Centre Gallery

POSTERS – 1

Posters should be displayed from Monday UNTIL NO LATER THAN 18:00, THURSDAY

BACTERIA

B-01 **Generation of a *Manduca sexta* larval midgut EST collection** Yannick Pauchet[1]; Heiko Vogel[2]; Paul Wilkinson[1]; David G. Heckel[2]; Richard H. ffrench-Constant[1], [1]University of Exeter in Cornwall, Penryn, UK; [2]Max Planck Institute for Chemical Ecology, Germany

B-02 **Understanding the interactions of two novel Cyt-toxins** Kara S. Giddings[1]; Andrew M. Wollacott[2], [1]Monsanto Company, Chesterfield MO, USA; [2]Monsanto Company, Cambridge MA, USA

B-03 **Variability in the *cadherin* gene in the European corn borer, *Ostrinia nubilalis* (Hübner)** Yolanda Bel[1]; Juan Ferré[1]; Baltasar Escriche[1], [1]University of Valencia, Spain

B-04 **Comparison of wild-type and mutant forms of Bt toxin Cyt1A in molecular dynamics simulations** Xiaochuan Li[1]; Dexuan Xie[2]; Peter Butko[3], [1]Boston University, Boston, MA USA; [2]University of Wisconsin, Milwaukee, WI, USA; [3]University of Maryland, Baltimore, MD, USA

B-05 **Chitinase profiles and insecticidal effects of bacteria originated from hazelnut pests** Zihni Demirbag[1]; Bahar A. Adem[1]; Kazım Sezen[1]; Remziye Nalcacioglu[1], [1]Karadeniz Technical University, Trabzon, Turkey

B-06 STU **Interaction between REPAT members, a family of pathogen induced proteins** Gloria Navarro-Cerrillo[1]; Juan Ferré[1]; Ruud A. de Maagd[2]; Salvador Herrero[1], [1]University of Valencia, Spain; [2]Plant Research International B.V., Wageningen University, The Netherlands

B-07 **Expression profiles of aminopeptidase genes in *Heliothis virescens* larvae exposed to Bt toxins** Omaththage P. Perera[1]; Anais S. Castagnola[2]; Juan Luis Jurat-Fuentes[2]; Craig A. Abel[1], [1]USDA-ARS, Stoneville, MS, USA; [2]University of Tennessee, Knoxville, TN, USA

B-08 STU ***Spodoptera exigua* gene expression profile in response to sublethal intoxication by a commercial *Bacillus thuringiensis* based product** Patricia Hernandez-Martinez[1]; Gloria Navarro-Cerrillo[1]; Ruud A. de Maagd[2]; Baltasar Escriche[1]; Salvador Herrero[1], [1]Universitat de Valencia, Spain; [2]Business Unit Bioscience Plant Research International B.V., Wageningen, The Netherlands

B-09 ***Drosophila* embryos as a novel system for testing insecticidal toxins *in vivo*** Andrea J. Dowling[1]; Isabella Vlisidou[2]; Nicholas R. Waterfield[2]; Richard H. ffrench-Constant[1]; William Wood[2], [1]University of Exeter in Cornwall, Falmouth, UK; [2]University of Bath, UK

B-10 **Study on Bt susceptibility and resistance mechanisms in the sugarcane borer, *Diatraea saccharalis*** Yu Cheng Zhu[1]; Xiaoyi Wu[2]; Yunlong Yang[2]; James Ottea[2]; Roger Leonard[2]; Craig A. Abel[1]; Fangneng Huang[2], [1]USDA-ARS, Stoneville, MS, USA; [2]Louisiana State University, Baton Rouge, USA

B-11 STU **Characterization of the *Heliothis virescens* midgut regenerative response upon treatment with *Bacillus thuringiensis* Cry1Ac toxin** Anaïs S. Castagnola[1]; Omaththage P. Perera[2]; Juan Luis Jurat-Fuentes[1], [1]University of Tennessee, Knoxville, TN, USA; [2]USDA-ARS, Stoneville, MS, USA

B-12 **High temperature could trigger rapid development of resistance to *Bt* toxin Cry1Ac and deltamethrin in *Plutella xylostella*** Ali H. Sayyed[1]; Neil Crickmore[1], [1]University of Sussex, Falmer, Brighton, UK

B-13 STU **Characterisation of novel resistance and cross-resistance to *Bacillus thuringiensis* crystal toxin** Paul R. Johnston[1]; Vidisha Krishnan[1]; Ruchir Mishra[1]; Ali H. Sayyed[1]; Neil Crickmore[1], [1]University of Sussex, Brighton, UK

B-14 STU **Characterization of the Cry41Aa parasporin** Vidisha Krishnan[1]; Stella Stamatopoulou[1]; Hideki Katayama[2]; Eiichi Mizuki[3]; Neil Crickmore[1], [1]University of Sussex, Brighton, UK; [2]Biotechnology and Food Research Institute, Fukuoka, Japan

B-15 STU **The efficacy of non-mosquitocidal Malaysian Bt isolates (Bt18) against three leukemic cell lines (CEM-SS, CCRF-SB and CCRF-HSB-2) and its mode of cell death** Chan K. Keong[1]; Nadarajah V. Devi[1]; Mohamed S. Mariam[1]; Abdullah Maha[2], [1]International Medical University, Kuala Lumpur, Malaysia; [2]Universiti Putra Malaysia, Selangor, Malaysia

B-16 STU **Identification of GAPDH as a putative receptor for a 68-kDa *Bacillus thuringiensis* parasporal protein cytotoxic against leukaemic cells** Kanakeswary K; VD Nadarajah; SM Mohammed, International Medical University, Kuala Lumpur, Malaysia

B-17 **Different mechanisms of action of *Bacillus thuringiensis* Cry1Ac toxin along the midgut of lepidopteran larvae** Silvia Caccia[1]; Ana Rodrigo-Simón[1]; Juan Ferré[1], [1]Universitat de València, Spain

B-18 STU **Study of two midgut aminopeptidases from *Ostrinia nubilalis* Hübner** Cristina M. Crava[1]; Yolanda Bel[1]; Barbara Manachini[2]; Baltasar Escriche[1], [1]University of Valencia, Valencia, Spain; [2]University of Palermo, Palermo, Italy

B-19 **Characterization of the interactions of *Bacillus thuringiensis* delta-endotoxins with the gut of the pea aphid, *Acyrthosiphon pisum* (Harris)** Huarong Li[1]; Bryony C. Bonning[1], [1]Iowa State University, Ames IA, USA

B-20 STU **Analysis of receptor-binding region for effective improvement of Cry1Aa insecticidal activity** Fumiaki Obata[1]; Madoka Kitami[1]; Yukino Inoue[1]; Takuya Kotani[1]; Yuko Harashima[1]; Chinatsu Morimoto[1]; Yasushi Hoshino[1]; Delwar M. Hossain[1]; Ryoichi Sato[1], [1]Tokyo University of Agriculture and Technology, Koganei, Tokyo, Japan

B-21 **Genetic stability of the putative marker *Bacillus thuringiensis* S76GFP^{+} expressing a green fluorescence protein (GFP) in the absence of selective pressure** Juliana C. de Orem[1]; Ana F. Parente[1]; Mariana T R Lira[1]; Tayana Kariya[1]; Isabela M M de Oliveira[1]; Marlene T. De-Souza[1], [1]Brasilia University, Brazil

B-22 STU **Construction of modified *Bacillus thuringiensis cry1Ac* genes based on *cry1-5* genes through multi site-directed mutagenesis** Hong Guang Xu[1]; Jong Yul Roh[1]; Jae Young Choi[2]; Hee Jin Shim[1]; Yong Wang[1]; Qin Liu[1]; Soo Dong Woo[3]; Byung Rae Jin[4]; Yeon Ho Je[1], [1]Seoul National University, Korea; [2]Research Institute for Agriculture and Life Sciences, Seoul National University, Korea; [3]Chungbuk National University, Korea; [4]Dong-A University, Korea

B-23 **Construction of a *Bacillus thuringiensis* engineered strain with high toxicity and broad insecticidal spectrum to Coleopteran by homologous recombination** Jingjing Liu[1,2]; Jie Zhang[2]; Changlong Shu[2]; Fuping Song[2]; Guixin Yan[2]; Dafang Huang[3], [1]Northeast Agricultural University, Harbin, China; [2]Chinese Academy of Agricultural Sciences, Beijing, China

B-24 **Engineered *Bacillus thuringiensis* 3A-HBF with insecticidal activity against Scarabaeidae and Chrysomelidae** Guixin Yan[1]; Changlong Shu[1]; Fuping Song[1]; Jingjing Liu[2]; Dafang Huang[2]; Jie Zhang[1], [1]Chinese Academy of Agricultural Sciences, Beijing, China; [2]Northeast Agricultural University, Harbin, China

B-25 **Studies on protease-resistant core form of *Bacillus thuringiensis* Cry1Ie toxin** Shuyuan Guo[1]; Yancai Zhang[1]; Jie Zhang[2]; Fuping Song[2]; Dafang Huang[3], [1]Beijing Institute of Technology, China; [2]CAAS, Beijing, China; [3]Chinese Academy of Agricultural Sciences, Beijing, China

B-26 STU **20kb DNA: What is it doing in Bt crystals?** Seher Fazal[1]; Christopher Jones[1]; Neil Crickmore[1], [1]University of Sussex, Brighton, UK

B-27 **Evidence of the involvement of the C-terminal portion of *Bacillus thuringiensis* Cry1Ac delta-endotoxin in crystallization** Slim Tounsi[1]; Mariam Dammak[1]; Samir Jaoua[1], [1]Centre of Biotechnology of Sfax, Sfax,Tunisia

B-28 ***Bacillus thuringiensis* serovar *thompsoni* HD542 crystal proteins: Solubilization, activation, and insecticidal activity** Samir Naimov[1]; Rumyana Boncheva[1]; Ruud deMaagd[2], [1]University of Plovdiv "Paisii Hilendarski", Bulgaria; [2]Plant Research International B.V, Wageningen University and Research Centre, The Netherlands

B-29 **Characterization of environmental isolates of *Bacillus thuringiensis* from northeastern Poland harbouring *vip3A* gene homologues** Izabela Swiecicka[1]; Dennis K. Bideshi[2]; Magdalena Czajkowska[1]; Sylwia Kotowicz[1], [1]University of Bialystok, Poland; [2]California Baptist University, Riverside, California USA

B-30 **Characterization of a novel Cry9Bb δ-endotoxin from *Bacillus thuringiensis*** Joseilde O. Silva-Werneck[1]; David J. Ellar[2], [1]Embrapa Recursos Genéticos e Biotecnologia, Brasília, Brazil; [2]University of Cambridge, UK

B-31 STU **Identification and cloning of novel *cry* genes from *Bacillus thuringiensis* strain Y41** Changlong Shu[1]; Xudong Su[1]; Jie Zhang[1]; Dafang Huang[2]; Fuping Song[1], [1]Institute of Plant Protection, and [2]Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing, P. R. China

B-32 STU **The characterization of novel Bt toxins** Zenas George[1]; Neil Crickmore[1], [1]University of Sussex, Falmer, Brighton, UK

B-33 STU **Identification of new *cry* genes of *Bacillus thuringiensis* through the use of a system of universal primers** Pedro A. Noguera[1]; Jorge E. Ibarra[1],[1]Departamento de Biotecnología y Bioquímica, CINVESTAV-IPN, Irapuato, Mexico

B-34 **Genetic diversity of *cry g*ene sequences of *Bacillus thuringiensis* strains analyzed by denaturing gradient gel electrophoresis** Corina M. Berón[1]; Macarena Pérez-Cenci[1]; Graciela L. Salerno[1], [1]Fundación para Investigaciones Biológicas Aplicadas (FIBA), Argentina

B-35 **Cyanogenesis in *Pseudomonas entomophila*: An entomopathogenic bacterium** Ben Ryall[1]; Hannah Nasser[1]; Dimitris Mossialos[2]; Huw D. Williams[1], [1]Imperial College London, UK; [2]University of Thessaly, Larissa, Greece

B-36 ***Bacillus thuringiensis*: Genetic diversity of Brazilian Lepidoptera specific isolates** Ana M. Guidelli-Thuler[1]; Janete A. Desidério Sena[1]; Irlan L. de Abreu[1]; Camila C. Davolos[1]; Sergio B. Alves[2]; Ricardo A. Polanczyk[3]; Fernando H. Valicente[4]; Manoel Victor F. Lemos[1], [1]Universidade Estadual Julio Mesquita Filho (UNESP Jaboticabal), Brasil; [2]Escola Superior de Agricultura Luiz de Queiroz (ESALQ/USP), Brasil; [3]Centro de Ciencias Agrarias - Universidade Federal do Espírito Santo, Brasil; [4]Empresa Brasileira de Pesquisa Agropecuária, Brasil

B-37 STU **Characterization of an endophytic *Bacillus thuringiensis* strain isolated from sugar cane** Marise T. Suzuki[1]; C. Sara Hernández-Rodríguez[1]; Welington L. de Araújo[2]; Juan Ferré[1], [1]Universitat de València, Spain; [2]Universidade de São Paulo, Brazil

B-38 **Electron-microscopic and genetic characterization of '*Rickettsiella tipulae*', an intracellular bacterial pathogen of the crane fly, *Tipula paludosa*** Regina G. Kleespies[1]; Andreas Leclerque[1], [1]Julius Kuehn-Institute, Germany

B-39 **Functional analysis of nematocidal protein Cry6Aa2 from *Bacillus thuringiensis*** Jun Cai; Xue-Zhao Liu; Yong-Qiang Jia; Bing Yan; Yue-Hua Chen; Yu Yuan, Nankai University, Tianjin, China

B-40 **Characterisation of two *Bacillus thuringiensis* subsp. *morrisoni* strains isolated from *Thaumetopoea pityocampa* Den. and Schiff., (Lep., Thaumetopoeidae)** Hatice Kati[1]; Ikbal A. Ince[1]; Kazim Sezen[2]; Şerife Isci[2]; Zihni Demirbag[2], [1]Giresun University, Turkey; [2]Karadeniz Technical University, Turkey

B-41 **Characterization of *Bacillus thuringiensis* strain collections from Spain and evaluation of their insecticidal activity against *Ceratitis capitata*** José Cristian Vidal-Quist[1]; Pedro Castañera[2]; Joel González-Cabrera[1], [1]Instituto Valenciano de Investigaciones Agrarias, Spain; [2]Centro de Investigaciones Biológicas, Madrid, Spain

B-42 **Susceptibility to *Bacillus thuringensis* of neonates and older larvae of *Tortrix viridiana* L. (Lepidoptera: Tortricidae) from a natural reserve** Barbara Manachini[1]; Filippo Castiglia[2], [1] University of Palermo, Italy; [2]Azienda Regionale Foreste Demaniali, Palermo, Italy

B-43 ***Bacillus thuringiensis* as a biological control agent for the red palm weevil, *Rhynchophorous ferrugineus* (Oliv.) (Coleoptera, Curculionidae)** Barbara Manachini; Paolo Lo Bue; Ezio Peri; Stefano Colazza, University of Palermo, Italy

B-44 **New strategy for isolating novel nematicidal crystal protein genes from *Bacillus thuringiensis* strain YBT-1518** Suxia Guo[1]; Donghai Peng[1]; Weiya Li[1]; Sisi Ji[1]; Pengxia Wang[1]; Ziniu Yu[1]; Ming Sun[1], [1]Huazhong Agricultural University, Wuhan, P.R. China

B-45 **Physiological characterization of accumulated poly-β-hydroxybutyrate(PHB) in *Bacillus thuringiensis*** Chen Deju[1]; Yan Jin[1]; Meng Ying[1]; Chen Shouwen[1]; Sun Ming[1]; Yu Ziniu[1],[1]Huazhong Agricultural University, Wuhan P. R. China

B-46 **Influence of different strategies of European corn borer (*Ostrinia nubilalis* Hübner) control on the content of contaminants in maize** Vladan Falta[1]; Jitka Stará[1]; František Kocourek[1]; Ludmila Slezáková[1]; Jana Hajšlová[2]; Vladimír Kocourek[2]; O. Lacina[2]; J. Honzíček[2]; Jana Tichá[2]; Alexandra Krplová[2]; Monika Gocieková[2], [1]Crop Research Institute, Prague, Czech Republic; [2]Institute of Chemical Technology Prague, Czech Republic

B-47 **Efficacy of different strategies of European corn borer (*Ostrinia nubilalis* Hübner) control in maize** Jitka Stará[1]; Vladan Falta[1]; František Kocourek[1]; Ludmila Slezáková[1], [1]Crop Research Institute, Prague, Czech Republic

B-48 **Identification of commercial BT-strains by molecular markers** Gian Paolo Barzanti[1]; Elena Cosi[1]; Pietro Rumine[1]; Pio F. Roversi[1], [1]C.R.A. - Centro di Ricerca per l'Agrobiologia e la Pedologia, Firenze, Italy

B-49 **Host plant preference of spider mites on Bt-expressing and control potatoes** Rostislav Zemek[1], [1]Institute of Entomology, Biology Centre AS CR, Czech Republic

B-50 **Interactions between Cry1Ac, Cry2Ab, and Cry1Fa *Bacillus thuringiensis* toxins in the cotton pests *Helicoverpa armigera* (Hübner) and *Earias insulana* (Boisduval)** Maria A. Ibargutxi[1]; Delia Muñoz[1]; Iñigo Ruiz de Escudero[1]; Primitivo Caballero[1], [1]Universidad Püblica de Navarra, Pamplona, Spain

B-51 **Development of the proteinaceous insecticide from a soil bacterium (*Bacillus thuringiensis)* using phage display** Delwar M. Hossain[1]; Takuya Kotani[1]; Chinatsu Morimoto[1]; Yuko Harashima[1]; Ryoichi Sato[1], [1]Tokyo University of Agriculture and Technology, Tokyo Japan

B-52 **Screening for more toxic δ-endotoxins of *Bacillus thuringiensis* for the management of *Spodoptera litura* in India** Venkatasamy Balasubramani[1, 2]; P. R. Johnston[2]; Neil Crickmore[2], [1]Tamil Nadu Agricultural University, Coimbatore, India; [2]University of Sussez, Brighton, UK

FUNGI

F-01 **Differential UV tolerance amongst spore-cell types of the entomopathogenic fungus *Beauveria* (*Cordyceps*) *bassiana*** Everton K. K. Fernandes[1]; Brett H. Kirkland[2]; Nemat O. Keyhani[2]; Chad A. Keyser[1]; Donald W. Roberts[1], [1]Utah State University, Logan, UT, USA; [2]University of Florida, Gainesville, FL, USA

F-02 STU **Effects of *Beauveria bassiana* on the bark beetle *Ips sexdentatus* and on its predator *Thanasimus formicarius*** Bernhardt M. Steinwender[1]; Rudolf Wegensteiner[1], [1]BOKU - University of Natural Resources and Applied Life Sciences, Vienna, Austria

F-03 **Pathogenicity of *Beauveria bassiana* and *Beauveria brongniartii* to the bark beetle *Ips typographus* L. (Coleoptera: Scolitidae)** M. Burjanadze[1], [1]Vasil Gulisashvili Forest Institute, Tbilisi, Georgia

F-04 **Scanning the virulence of sixty isolates of *Beauveria* spp. and *Engyodontium albus* to the cattle tick *Boophilus microplus*** Everton K. K. Fernandes[1]; Isabele C. Angelo[1]; Thiago C. Bahiense[1]; Donald W. Roberts[2]; Vania R. E. P. Bittencourt[1], [1]Curso de Pos Graduacao em Ciencias Veterinarias, Brazil; [2]Utah State University, Logan, USA

F-05 **The first record of *Beauveria bassiana* (Deuteromycetes) on the hibernating pupae of *Cameraria ohridella* (Lepidoptera: Gracillariidae)** Eva Prenerova[1]; Rostislav Zemek[2]; Frantisek Weyda[2], [1]Laboratory of Plant Protection Olesna, Czech Republic; [2]Institute of Entomology, Biology Centre AS CR, Czech Republic

F-06 ***Metarhizium anisopliae* microsclerotia: Production and bioefficacy** Stefan T. Jaronski[1]; Mark A. Jackson[2], [1]USDA ARS Sidney MT, USA; [2]USDA ARS Peoria IL, USA

F-07 **Morphological characteristics and insect virulence bioassays of two high temperature adapted *M. anisopliae* strains** Eudes de Crecy[1]; Stefan Jaronski[2]; Nemat O. Keyhani[3], [1]Evolugate LLC, Gainesville, FL, USA; [2]USDA ARS, Sidney MT, USA; [3]University of Florida, Gainesville, FL, USA

F-08 **Variability and identification of *Metarhizium* varieties and species based on heat tolerance, cold activity and molecular analysis** Everton K. K. Fernandes[1]; Chad A. Keyser[1]; Drauzio E. N. Rangel[1]; Mark P. Miller[1]; Donald W. Roberts[1], [1]Utah State University, Logan, UT, USA

F-09 STU **Comparison of new and commercial *Metarhizium* isolates based on multiple traits** Chad A. Keyser[1]; Everton K. K. Fernandes[1]; Drauzio E. N. Rangel[1]; Donald W. Roberts[1], [1]Utah State University, Logan, UT, USA

F-10 **Novel delivery of the fungus *Paecilomyces fumosoroseus* (Deuteromycotina: Hyphomycetes) for managing the Asian citrus psyllid (Psyllidae: Hemiptera): Laboratory investigation** Pasco B. Avery[1]; Wayne B. Hunter[2]; David G. Hall[2]; Mark A. Jackson[3]; Michael E. Rogers[1]; Charles A. Powell[1], [1]University of Florida, Ft. Pierce, FL, USA; [2]USDA, ARS, Ft. Pierce, FL, USA; [3]USDA, ARS, Peoria, IL, USA

F-11 **Potential of topical application, leaf residue and soil drench of fungus *Paecilomyces fumosoroseus* (Deuteromycotina: Hyphomycetes) for killing citrus weevil: Laboratory and greenhouse investigation** Pasco B. Avery[1]; Wayne B. Hunter[2]; David G. Hall[2]; Mark A. Jackson[3]; Michael E. Rogers[1]; Charles A. Powell[1], [1]Univeristy of Florida-IFAS, Ft. Pierce, FL, USA; [2]USDA, ARS, Ft. Pierce, FL, USA; [3]USDA, ARS, Peoria, IL, USA

F-12 **Growth inhibition and revitalization of mycelia of *Paecilomyces tenuipes*, an entomopathogenic fungus** Sung Hee Nam[1]; Chun Ru Li[2]; In Pyo Hong[1]; Kyu Byoung Sung[1]; Ji Young Choi[1]; Seok Woo Kang[1]; Mei-Zhen Fan[1]; Zeng-Zhi Li[1],[1]National Institute of Agricultural Science and Technology, Suwon, Korea; [2]Anhui Agricultural University, Hefei, P.R. China

F-13 **Reassessment of vegetative compatibility groups (VCGs) in Japanese isolates of *Lecanicillium* spp.(*Verticillium lecanii*)** Masanori Koike[1]; Midori Sugimoto[1]; Daigo Aiuchi[1], [1]Obihiro University, Hokkaido, Japan

F-14 **Ultraviolet light protection of *Lecanicillium attenuatum* with titanium dioxide** Jeong Jun Kim[1]; Drauzio E.N. Rangel[2]; Donald W. Roberts[2]; Dong-ro Choi[1],[1]NIAST, Suwon, Korea; [2]Utah State University, Logan UT, USA

F-15 STU ***Lecanicillium* spp. (= *Verticillium lecanii*) penetrate into *Trialeurodes vaporariorum* egg** Daigo Aiuchi[1]; Sayaka Horie[2]; Masanori Koike[2], [1]Iwate University, Morioka, Japan; [2]Obihiro Univ. of Agriculture and Veterinary Medicine, Japan

F-16 STU **Preventive application to control cotton aphids and greenhouse whiteflies by *Verticillium lecanii* (=*Lecanicillium* spp.)** Sayaka Horie[1]; Daigo Aiuchi[2]; Toshihiro Watanabe[1]; Masanori Koike[1],[1]Obihiro University of Agriculture and Veterinary Medicine, Hokkaido, Japan; [2] Iwate University, Morioka, Japan

F-17 STU **Sporulation of *Verticillium lecanii* (=*Lecanicillium* spp.) on cotton aphid cadaver in different humidity conditions** Toshihiro Watanabe[1]; Daigo Aiuchi[2]; Masanori Koike[1], [1]Obihiro University of Agriculture and Veterinary Medicine, Hokkaido, Japan; [2]Iwate University, Japan

F-18 **Beneficial associations between *Pandora neoaphidis* and noncrop plants inhabiting lettuce field margins** Beatriz M. Diaz[1]; José Dorado[1]; Saioa Legarrea[1]; Alberto Fereres[1], [1]CCMA-CSIC, Madrid, Spain

F-19 **Spatial distribution of aphids infected by *Pandora neoaphidis* and aphidophagous syrphids in lettuce crops** Beatriz M. Diaz[1]; Saioa Legarrea[1]; Ignacio Morales[1]; María A. Marcos-García[2]; Alberto Fereres[1],[1]CCMA-CSIC, Madrid, Spain; [2]CIBIO, Universidad de Alicante, Alicante, Spain

F-20 **Transmission of *Pandora neoaphidis* in the presence of co-occurring arthropods** Jason Baverstock[1]; Katherine E. Baverstock[1]; Suzanne J. Clark[1]; Judith K. Pell[1],[1]Rothamsted Research, Harpenden, Hertfordshire, UK

F-21 **Molecular characterization of entomopathogenic fungi using microsatellite markers** Surendra K. Dara[1,4]; Michael R. McGuire[2,5]; Mauricio Ulloa[2]; Harry K Kaya[3], [1]Shafter Research and Extension Center-UC Davis, CA, USA; [2]USDA-ARS, Shafter, CA, USA; [3]University of California, Davis, CA, USA; Current address: [4]CertisUSA, Wasco, CA, USA; [5]USDA-ARS-NRRC, Fort Collins, CO, USA

F-22 **Isolation and characterization of a photolyase gene from the entomopathogenic fungi *Beauveria bassiana*** Lady C. Rosero[1]; Sandra Valdez[1]; Luz M. Escobar[1]; Narmer F. Galeano[1]; Carmenza E. Gongora[1], [1]National Centre of Coffee Research CENICAFE-FNC, Caldas, Colombia

F-23 **Identification of transcripts with increased expression during conidiogenesis of the entomopathogenic fungus *Metarhizium anisopliae*** Everaldo R. Marques[1]; Sérgio H. Silva[1]; Donald W. Roberts[2]; Gilberto U. L. Braga[1], [1]Universidade de São Paulo, Brasil; [2]Utah State University, Logan, UT, USA

F-24 **Avoidance of entomopathogenic fungi by insect predators** Nicolai V. Meyling[1]; Emma Ormond[2]; Helen E. Roy[3]; Judith K. Pell[4], [1]University of Copenhagen, Denmark; [2]Anglia Ruskin University, Cambridge, UK; [3]NERC Centre for Ecology and Hydrology, Cambridgeshire, UK; [4]Rothamsted Research, Plant and Invertebrate Ecology Department, Harpenden, Hertfordshire, UK

F-25 **Isolation of entomopathogenic fungi from soil collected from western United States** Everton K. K. Fernandes[1]; Chad A. Keyser[1]; Drauzio E. N. Rangel[1]; R. Nelson Foster[2]; Donald W. Roberts[1], [1] Utah State University, Logan, UT, USA; [2]USDA/APHIS/PPQ/CPHST Lab, Phoenix, AZ, USA

F-26 **Survey for entomopathogenic fungi from *Rhynchophorous ferrugineus* (Oliv.) (Coleoptera, Curculionidae)** Barbara Manachini, Sandra Marineo, Franco Palla, University of Palermo, Italy

F-27 STU **Induction of defense-related genes in banana (*Musa* spp.) by endophytic *Fusarium oxysporum*** Pamela Paparu[1]; Thomas Dubois[2]; Daniel Coyne[2]; Claire Munro[1]; Altus Viljoen[3], [1]University of Pretoria, South Africa; [2]International Institute of Tropical Agriculture, Kampala, Uganda; [3]University of Stellenbosch, South Africa

F-28 STU **Observations of fungal disease in the giant willow aphid (*Tuberolachnus salignus*): Is it a new species of *Neozygites*?** Gudbjorg Aradottir[1,2]; Richard Harrington[2]; Angela Karp[2]; Steve Hanley[2]; Ian Shield[2]; William Macalpine[2]; Matilda Collins[2]; Simon Leather[2]; Judith Pell[2], [1]Rothamsted Research, Harpenden, Hertfordshire, UK; [2]Imperial College London, Ascot, UK

12:30–14:00 **LUNCH** Rootes Restaurant

13:30-18:30 **EXCURSION**

19:00-23:00 **BBQ** including presentation of 5K awards and Auction

WEDNESDAY - 6 August

Symposium (Bacteria Division) Wednes., 8:00–10:00. Arts C. Theatre

Entomopathogenic Bacteria Other than *Bacillus*

Organizers/Moderators: Christina Nielsen-LeRoux and Juan-Luis Jurat-Fuentes.

8:00 **90** ***Drosophila* host defence against *Pseudomonas entomophila*** Onya Opota[1]; Bruno Lemaitre[1], [1]Ecole Polytechnique Federale de Lausanne, Switzerland

8:30 **91** **Virulence determinants of *Yersinia entomophaga* MH96: a genomic perspective.** Mark R H Hurst[1]; Regina Shaw[2]; William G. Farmerie [2]; Anette Becher[3],[1]AgResaerch, Bioprocessing and Biosecurity, Canterbury, New Zealand; [2]University of Florida, Gainesville, FL, USA; [3]AgResearch, Invermay, New Zealand

9:00 **92** **Insecticidal toxins from *Photorhabdus*: Comparative genomics and Rapid Virulence Annotation (RVA)** Richard H. ffrench-Constant[1]; Stewart Hinchliffe[1]; Michelle Hares[1]; Andrea J. Dowling[1]; Nicholas Waterfield[2]; Isabella Vlisidou[2]; Maria Sanchez Contreras[2], [1]University of Exeter in Cornwall, Penryn, UK; [2]University of Bath, UK

9:30 **93** **Pathogenesis of *Serratia entomophila* (Enterobacteriaceae) towards the New Zealand grass grub *Costelytra zealandica*.** Trevor A. Jackson[1]; Sean M. Marshall[1]; Mark R.H. Hurst[1]; Drion G. Boucias[2]; Heather S. Gatehouse[3]; John C. Christeller[3], [1]AgResearch, Canterbury, New Zealand; [2]University of Florida, Gainesville, FL, USA; [3]Horticulture and Food Research Institute, New Zealand

Symposium (Microsoridia Division) Wednes., 8:00–10:00. SS020

Microsporidia of Aquatic Arthropods

Organizer/Moderator: Regina Kleespies

8:00 **94** **Microsporidian parasite of caddis flies (Trichoptera) with comment to phylogeny and classification of Microsporidia in general** Miroslav Hyliš[1], [1]Charles University, Prague, Czech Republic

8:20 **95** **Evolutionary interactions between microsporidia and their hosts: Lessons from an ancient lake** Judith E. Smith[1]; Qui Yang[1]; Ravil M. Kamaltynov[2]; Dmitry Y. Sherbakov[3], [1]Leeds University, UK; [2]Siberian Branch of Russian Academy of Sciences, Irkutsk, Russia

8:40 **96** **Microsporidia in freshwater Amphipods: an overview and an example** Remi A. Wattier[1]; Karolina Bacela[1]; Thierry Rigaud[1], [1]Université de Bourgogne, Dijon, Burgundy, France

9:00 **97** **Coevolutionary dynamics of host-parasite interactions in natural *Daphnia* populations** Ellen Decaestecker[1] [1]K.U.Leuven - Campus Kortrijk, Belgium

9:20 **98** **Epizootiological studies of *Amblyospora camposi* (Microsporidia: Amblyosporidae) in *Culex renatoi* (Diptera:Culicidae) and *Paracyclops fimbriatus fimbriatus* (Copepoda:Cyclopidae) in a bromeliad habitat** Victoria Micieli[1]; James J. Becnel[2]; Gerardo A. Marti[1]; María C. Tranchida[1]; Juan J. García[3], [1]Centro de Estudios Parasitológicos y de Vectores- CEPAVE (UNLP-CONICET), Argentina; [2]USDA, ARS, Gainesville, FL, USA

9:40 **99** **Intranuclear microsporidians in crustaceans: The genus Enterospora** Grant D. Stentiford[1],[1]Centre for Environment, Fisheries and Aquaculture Science, Weymouth, Dorset, UK

Contributed Papers Wednesday, 8:00-10:00. SS021

FUNGI 2

Moderator: Surendra Dara.

8:00 **100** **Genetic analysis of conidiation mutants in *Metarhizium anisopliae* derived by *Agrobacterium*-mediated mutagenesis** Farah-Jade Dryburgh[1]; Weiguo Fang[2]; Raymond J. St. Leger[2]; Michael J. Bidochka[1],[1]Brock University, ON, Canada [2]University of Maryland, College Park, Maryland, USA

8:15 **101** **Directed adaptation of *Metarhizium anisopliae* to cockroach cuticle** Eudes de Crecy[1]; Nemat O. Keyhani[2], [1]Evolugate LLC, Gainesville, FL, USA; [2]University of Florida, Gainesville, FL, USA

8:30 **102** **The effect of tick species and stages on the pre-penetration steps of the entomopathogenic fungi, *Metarhizium anisopliae*** Galina Gindin[1]; Dana Ment[1]; Asael Rot[2]; Itamar Glazer[1]; Michael Samish[3],[1]The Volcani Center, (ARO), Bet Dagan, Israel; [2]Kimron Veterinary Institute, Bet Dagan, Israel

8:45 **103** **A proteomic approach to the identification of proteins differentially expressed in the conidia and mycelium of the entomopathogenic fungus *Metarhizium anisopliae*** Sérgio H. Silva[1]; Bruno H. R. Barros[1]; Everaldo R. Marques[1]; Ana Patrícia Yatsuda[1]; Donald W. Roberts[2]; Gilberto U. L. Braga[1], [1]Universidade de São Paulo, Brazil; [2]Utah State University, Logan, UT, USA

9:00 **104** **Transcript analysis of the entomopathogen *Beauveria bassiana* during the infection process on the coffee berry borer** Javier G. Mantilla; Sandra M. Idarraga; Alvaro L. Gaitán; Carmenza E. Góngora, National Centre of Coffee Research CENICAFE-FNC, PlanAlto, Chinchiná, Caldas, Colombia

9:15 **105 STU** **Alkane degradation by *Beauveria bassiana*: Gene expression analysis of cytochrome P450 monooxigenases** Nicolas Pedrini[1,2]; Patricia Juárez[1]; Nemat O. Keyhani[2], [1]Instituto de Investigaciones Bioquímicas de La Plata (CCT CONICET-UNLP), Argentina; [2]University of Florida, Gainesville, FL, USA

9:30 **106 STU** **May *Beauveria bassiana* secreted proteins be virulence factors?** Almudena Ortiz-Urquiza[1]; Laura Riveiro-Miranda[1]; Cándido Santiago-Álvarez[1]; Enrique Quesada-Moraga[1], [1]University of Córdoba, Spain

9:45 **107** **Live cell imaging of endocytosis and membrane properties of *Beauveria bassiana in vitro* and hemolymph derived cells** Michael W. Lewis[1]; Ines V. Robalino[1]; Nemat O. Keyhani[1], [1]University of Florida, Gainesville, FL, USA

Contributed Papers Wednesday, 8:00-9:45. Arts C. Conf. Rm.

VIRUSES 3

Moderators: Doreen Winstanley and Rollie Clem.

8:00 **108** **Deletion of the *egt* gene reduces within-host competitive fitness** Mark Zwart[1]; Wopke van der Werf[1]; Monique van Oers[1]; Lia Hemerik[1]; Jan van der Lent[1]; Arjan G. M. de Visser[1]; Just M. Vlak[1]; Jenny S. Cory[2],[1]Wageningen University, The Netherlands; [2]Algoma University College, Sault Ste. Marie, ON, Canada

8:15 **109 STU** **Characterization of climbing behavior gene in recombinant baculoviruses** Matthew R. Gardner[1]; James M. Slavicek[2]; Scott M. Geib[1]; Kelli Hoover[1],[1]Pennsylvania State University, USA; [2]USDA Forest Service, Delaware, OH, USA

8:30 **110 STU** **Conservation of DNA photolyase genes in plusiine nucleopolyhedroviruses** Fang Xu[1]; Just M. Vlak[1]; Monique Van Oers[1], [1]Wageningen University, The Netherlands

8:45 **111** ***Chrysodeixis chalcites* nucleopolyhedrovirus encodes an active DNA photolyase** Monique M. van Oers[1]; Margit H. Lampen[1]; Monika I. Bajek[2]; Fang Xu[1]; Just M. Vlak[1]; André P.M. Eker[2], [1]Wageningen University, The Netherlands; [2]Erasmus University Medical Centre, Rotterdam, the Netherlands

9:00 **112 STU** **Anti-viral defenses in gypsy moth larvae: Evidence for the importance of immune responses within the host** James R. McNeil[1]; Diana Cox-Foster[1]; Lauren Ellis[1]; Kelli Hoover[1], [1] Penn State University, University Park, PA, USA

9:15 **113** **Baculovirus infection of immunosuppressed *S. littoralis* as a tool to study the lepidopteran anti-viral response** Nor Chejanovsky[1]; Haddassah Rivkin[1]; Irit Ornan[1], [1]Entomology The Volcani Center, Bet Dagan, Israel

9:30 **114** **An Ac*M*NPV *fgf* knockout mutant exhibits a defect in systemic infection of *Trichoplusia ni* larvae** John C. Means[1]; A. Lorena Passarelli[1], [1]Kansas State University, Manhattan KS, USA

10:00–10:30 **BREAK** Arts Centre Gallery

Symposium (Div. of Nematodes) Wednesday, 10:30-12:30. SS020

Entomopathogenic Nematode Application Technology in IPM

Organizers/Moderators: Claudia Dolinski and David Shapiro-Ilan.

10:30 **115** **Current status in application technology** Peters Arne[1], [1]e-Nema, Raisdorf, Germany

11:00 **116** **Cadaver application** Claudia Dolinski[1]; Edwin E. Lewis[2]; David Shapiro-Ilan[3], [1]Universidade Estadual do Norte Fluminense Darcy Ribeiro, Brazil; [2]Univeristy of California Davis, CA, USA; [3]USDA-ARS, Byron, GA, USA

11:30 **117** **Above ground and cryptic habitats application** Richard Glass[1]; Keith F. Walters[1], [1]Central Science Laboratory, Sand Hutton, York, UK

12:00 **118** **Enhancing post-application survival of entomopathogenic nematodes** Lerry Lacey[1], USDA-ARS, Yakima Agricultural Research Laboratory, Wapato, WA, USA

Contributed Papers Wednesday, 10:30-12:15. Arts C. Theatre

BACTERIA 2

Moderator: David Pauron.

10:30 **119** **A novel gene cluster encoding an insect toxin in plant-associated strains of *Pseudomonas fluorescens*** Maria Pechy-Tarr[1]; Denny J. Bruck[2]; Monika Maurhofer[3]; Esther Fischer[4]; Christelle Vogne[1]; Jurg Grunder[4]; Joyce E. Loper[2]; Christoph Keel[1], [1]University of Lausanne, Switzerland; [2]USDA-ARS Corvallis OR, USA; [3]Swiss Federal Institute of Technology, Zurich, Switzerland; [4]University of Applied Sciences HSW, Switzerland

10:45 **120 STU** **Functional characterisation of a cell cycle inhibiting factor (CIF) in the entomopathogenic bacteria *Photorhabdus*** Carolina Varela Chavez[1]; Frédéric Taïeb[2]; Grégory Jubelin[2]; Gabriel Courties[1]; Alain Givaudan[1]; Eric Oswald[2]; Jean-Michel Escoubas[1]; Robert Zumbihl[1], [1]Université Montpellier 2, France; [2]UMR, Toulouse, France

11:00 **121 STU** **Secondary lipid A acylation and extrusion by efflux pumps are two potential mechanisms of resistance to anti-microbial peptides in the entomopathogenic bacterium *Photorhabdus luminescens*** Ziad Abi Khattar[1]; Anne Lanois[1]; Sylvie Pagès[1]; Mireille Kallassy[2]; Sophie Gaudriault[1]; Alain Givaudan[1], [1]Université Montpellier II, France; [2]Université Saint-Joseph, Beirut, Lebanon

11:15 **122 STU** **Structural studies of toxin complexes** Michelle C. Hares[1]; Corinne Smith[2]; Sarah Lee[3]; Richard H. ffrench-Constant[1], [1]University of Exeter, Penryn, Cornwall, UK; [2]University of Warwick, Coventry, UK; [3]University of Warwick, Warwick HRI, Wellsbourne, Warwick, UK

11:30 **123 STU** **Interaction between Cry1Ab oligomer and their receptors alkaline phosphatase and aminopeptidase-N from *Manduca sexta*** Iván Arenas[1]; Alejandra Bravo[1]; Mario Soberón[1]; Isabel Gómez[1], [1]Biotechnology Institute, UNAM, Cuernavaca, Morelos, México

11:45 **124 STU** **Cry1Ab oligomeric structure elucidated by transmission electron microscopy** Nuria Jiménez-Juárez[1]; Liliana Pardo-Lopez[1]; Rosana Sánchez[1]; Carlos Muñoz-Garay[1]; Christos Savva[2]; Andreas Holzenberg[2]; Mario Soberón[1]; Alejandra Bravo[1], [1]Instituto de Biotecnología UNAM, Morelos, México; [2]Texas A&M University, USA

12:00 **125** **Effects of amino acid substitutions in a loop connecting beta-6 and beta-7 of a cytolytic toxin from *Bacillus thuringiensis***
Boonhiang Promdonkoy[1]; Wanwarang Pathaichindachote[1]; Duong N. Hoang[1]; Noppawan Nounjan[1]; Patcharee Promdonkoy[1]; Chartchai Krittanai[2]; Mongkon Audtho[1], [1]National Center for Genetic Engineering and Biotechnology, Pathumthani, Thailand; [2]Mahidol University, Thailand

Contributed Papers Wednesday, 10:30-12:15. SS021

MICROBIAL CONTROL 2

Moderators: John Vandenberg and Steve Wraight.

10:30 **126** ***Beauveria bassiana* as an artificial endophyte in tissue-cultured banana (*Musa* spp.) plants: A novel way to combat the banana weevil *Cosmopolites sordidus***
Thomas Dubois[1]; Juliet Akello[1]; Daniel Coyne[1]; Samuel Kyamanywa[2], [1]International Institute of Tropical Agriculture, Kampala, Uganda; [2]Makerere University, Kampala, Uganda

10:45 **127** **Assessing the field persistence of introduced *Beauveria bassiana* GHA for emerald ash borer control by bioassay, culture on selective medium and real-time PCR**
Louela A. Castrillo[1]; Michael H. Griggs[2]; Houping Liu[3]; Leah S. Bauer[4]; John D. Vandenberg[2],[1]Cornell University, Ithaca, NY, USA; [2]USDA ARS, Ithaca, NY, USA; [3]Michigan State University, East Lansing, MI, USA; [4]USDA Forest Service, East Lansing, MI, USA

11:00 **129** **Improving efficacy of *Beauveria bassiana* foliar treatments against Colorado potato beetle via manipulation of spray-application parameters.**
Stephen P. Wraight[1]; Mark E. Ramos[1], [1]USDA-ARS, Ithaca, NY, USA

11:15 **130 STU** **Effect of storage conditions on the pathogenicity of entomopathogenic fungi *Beauveria bassiana* to control whitefly *Bemisia tabaci***
Aref H. Olleka[1]; Shun-xiang Ren[1], [1]South China Agriculture University, Guangzhou, China

11:30 **131 STU** **Effects of production media and fertilisers on persistence and virulence of *Beauveria bassiana* F418 strain and a transformant *gfp* F418 tr1 in soil**
Céline L. Blond[1]; Michael Brownbridge[1]; Travis R. Glare[1]; Hayley J. Ridgway[2]; R. Bruce Chapman[2], [1]AgResearch, New Zealand; [2]Lincoln University, New Zealand

11:45 **132** **Effect of preying on *Metarhizium anisopliae* - infected onion thrips larvae on some behavioural parameters of *Orius albidipennis***
Hamid-Reza Pourian[1]; Reza Talaei-Hassanloui[1]; Reyhaneh Ezzati-Tabrizi[1]; Aziz Kharazi-Pakdel[1], [1]University of Tehran, Iran

12:00 **133** **Assessing potential effects of the *Beauveria brongniartii* biological control agent on fungal community structures in soil microcosms**
Juerg Enkerli[1]; Kaspar Schwarzenbach[1]; Franco Widmer[1], [1]Agroscope Reckenholz-Taenikon Research Station ART, Zurich, Switzerland

Contributed Papers Wednesday, 10:30-12:30. Arts C. Conf. Rm

VIRUSES 4

Moderators: Rosemary Hails and Kelli Hoover.

10:30 **134** **Characterizing the physical state of covert or persistent baculovirus genomes in insect hosts**
Mark S. Hussey[1]; Rosa M. Murillo[1]; Rosie S. Hails[1]; Robert D. Possee[1], [1]CEH, Oxford, UK

10:45 **135** **Virus reactivation in *Spodoptera exigua* laboratory culture**
Rosa M. Murillo[1]; Hussey Mark[1]; Rosie S. Hails[1]; Robert D. Possee[1], [1]CEH, Oxford, UK

11:00 **136** **Vertical transmission and persistent infection of NPVs in Eastern Spruce Budworm**
Elizabeth M. Kemp[1,2]; David T. Woodward[2,3]; Jenny S. Cory[1,2], [1]Algoma University College, Sault Ste Marie, ON, Canada; [2]Great Lakes Forestry Centre, Canadian Forest Service, Sault Ste. Marie, ON, Canada; [3]University of Guelph, ON, Canada

11:15 **137** **Within-host dynamics and virulence of co-inoculated baculovirus isolates: Evidence of synergism and antagonism**
David T. Woodward[1,2]; Jenny S. Cory[2,3], [1]University of Guelph, ON, Canada; [2]Canadian Forest Service, Sault Ste. Marie, ON, Canada; [3]Algoma University College, Sault Ste. Marie, ON, Canada

11:30 **138 STU** **Aggregation and infection risk in Lepidoptera**
Joanna C. McTigue[1]; Steve M. Sait[2]; Rosie S. Hails[1], [1]Centre for Ecology and Hydrology, Oxford, UK; [2]University of Leeds, Leeds, UK

11:45 **139 STU** **Resistance to the CpGV: Improved efficiency by selection pressure on resistant hosts**
Marie Berling[1]; Miguel Lopez-Ferber[1]; Christine Blachère-Lopez[1]; Benoît Sauphanor[2]; Antoine Bonhomme[3], [1]EMA - centre LGEI, Ales, France; [2]INRA, Avignon, France; [3]NPP (Arysta LifeScience), Pau, France

12:00 **140** **Real-time PCR analysis of a mixed infection of granulovirus and nucleopolyhedrovirus from *Adoxophyes orana***
Sally L. Hilton[1]; Gary Keane[1]; Doreen Winstanley[1], [1]Warwick HRI, University of Warwick, Wellesbourne, Warwickshire, UK

12:15 **141** **The physical association of genetically distinct nucleocapsids contributes to the maintenance of nucleopolyhedrovirus diversity**
Gabriel Clavijo[1]; Oihane Simon[1]; Delia Muñoz[1]; Trevor Williams[2]; Miguel Lopez-Ferber[3]; Primitivo Caballero[1], [1]Universidad Publica de Navarra, Spain; [2]Instituto de Ecología AC, Veracruz Mexico; [3]Ecole des Mines d'Alès, France

Student Workshop Wednesday, 12:00-14:00. Chancellors, Rootes

Spreading the word: Skills for Communicating Science and Getting it Funded

Organizers/Moderators: Onya Opota and Patricia Stock.

12:00 **142** **Delivering oral presentations**
Brian A. Federici[1], [1]University of California-Riverside, Riverside, CA, USA

12:30 **143** **Editing and reviewing scientific manuscripts**
John D. Vandenberg[1], [1]USDA-ARS, Ithaca, New York, USA

13:00 **144** **Strategies for writing successful grant applications**
Peter J. Krell[1], [1]University of Guelph, Canada

13:30 **145** **What funding agencies want: Tips for getting your research funded**
S. Patricia Stock[1], [1]University of Arizona, USA

12:30–14:00 **LUNCH** Rootes Restaurant

Symposium (Cross-Divisional) Wednesday, 14:00-16:00. SS021

Pathogens of Bees

Organizer/Moderator: Ingemar Fries.

14:00 **146** **New insights into AFB pathogenesis**
Dominique Yue[1]; Anne Fünfhaus[1]; Ainura Ashiralieva[1]; Elke Genersch[1], [1]Institute for Bee Research, Hohen Neuendorf, Germany

14:24 **147** ***Nosema*** **in bumble bees: Steps towards understanding** Mark J.F. Brown[1], [1]University of Dublin Trinity College, Ireland

14:48 **148** **Sexual transmission of deformed wing virus in honeybees** Joachim R. de Miranda[1]; Ingemar Fries[2],[1]Queen's University Belfast, Northern Ireland; [2]Swedish University of Agricultural Sciences, Uppsala, Sweden

15:12 **149** **Epizootiological aspects of chalkbrood infections in the alfalfa leafcutting bee** Rosalind James[1], [1]USDA, ARS, Logan, UT, USA

15:36 **150** **Co-evolution of mites and social honeybees in Asia** Denis L. Anderson[1],[1]CSIRO Entomology, Canberra, Australia

Contributed Papers Wednesday, 14:00-16:00. Arts C. Theatre

BACTERIA 3

Moderator: Neil Crickmore.

14:00 **151** **Specificity of *Bacillus thuringiensis* delta-endotoxins: A review, finally…** Kees van Frankenhuyzen[1]; Carl Nystrom[1], [1]Canadian Forest Service, Great Lakes Forestry Centre, Sault Ste. Marie, Ontario, Canada

14:15 **152** **Gut flora not required for pathogenicity in *Bacillus thuringiensis* infecting diamondback moth** Ben Raymond[1]; Michael B. Bonsall[1], [1]Oxford University, Oxford, UK

14:30 **153** **Pathogenesis of *Bacillus thuringiensis* subsp. *kurstaki* in spruce budworm and gypsy moth** Kees van Frankenhuyzen[1]; Yuehong Liu[1], [1]Natural Resources Canada, Canadian Forest Service, Great Lakes Forestry Centre, Sault Ste. Marie, Ontario, Canada

14:45 **154 STU** **Distinct changes in immune system are associated with *Bt* exposure in *Bt*-resistant and *Bt*-susceptible *Trichoplusia ni* colonies** Jerry D. Ericsson[1]; Alida F. Janmaat[2]; Richard M. Plunkett[1]; Judith H. Myers[3]; Carl Lowenberger[1], [1]Simon Fraser University, Burnaby, BC, Canada; [2]University College of the Fraser Valley, Abbotsford, BC, Canada; [3]University of British Columbia, Vancouver, BC, Canada

15:00 **155 STU** **Characterization of intracellular response in mosquitoes to *Bacillus thuringiensis* Cry11Aa toxin** Angeles Cancino-Rodezno[1], Roberto Villaseñor[1], Mario Soberón[1], Sergio Encarnación[2], Humberto Lanz[3], Ivonne Castro[3], Juan Luis Jurat-Fuentes[4] and Alejandra Bravo[1], [1]Instituto de Biotecnología UNAM, Cuernavaca, México; [2]Centro de Ciencias Genómicas UNAM, Cuernavaca, México; [3]Instituto Nacional de Salud Pública, Cuernavaca, México; [4]University of Tennessee, Knoxville, TN, USA

15:15 **156 STU** **Kinetics of microbial degradation and chemical fixation of Cry 1Aa Bt toxin in various soils** Nordine Helassa[1]; Arij M'Charek[2]; Gabrielle Daudin[1]; Sylvie Noinville[3]; Philippe Déjardin[4]; Hervé Quiquampoix[1]; Siobhan Staunton[1],[1]INRA - Biogéochimie du Sol et de la Rhizosphère, Montpellier, France; [2]Faculté des Sciences de Tunis - Tunisie; [3]CNRSThiais, France; [4]CNRS - Institut Européen des Membranes, Montpellier, France

15:30 **157 STU** **The *ger* genes of pBtoxis are responsible for the alkaline-activation of germination in *Bacillus thuringiensis* subsp. *Israelensis*** Mostafa Abdoarrahem[1]; Colin Berry[1],[1]Cardiff University, UK

15:45 **158 STU** **Laboratory-selected Cry1Ac-resistant *Helicoverpa zea* (Lepidoptera: Noctuidae) cannot survive on *Bt* cotton: Implication of potential synergistic interactions of Cry1Ac and gossypol** Konasale J. Anilkumar[1]; William J. Moar[1], [1]Auburn University, AL, USA

Contributed Papers Wednesday, 14:00-15:30. SS020

NEMATODES 3

Moderator: Ralf-Udo Ehlers.

14:00 **159** **Are there differences in dispersal, infectivity and sex ratio between early or late emerging infective juveniles of *Steinernema carpocapsae?*** Aki Fujimoto[1]; Gulumser Cobanoglu[2]; Ed E. Lewis[3]; Harry K. Kaya[3],[1]Kumiai Chemical Industry Co, Shizuoka, Japan; [2]Hacettepe University , Ankara, Turkey; [3]University of California, Davis, CA, USA

14:15 **160** **Male *Steinernema longicaudum* do not sexually mature in the absence of female** Lemma Ebssa[1]; Christine T. Griffin[1], [1]National University of Ireland Maynooth, Co. Kildare, Ireland

14:30 **161 STU** **Habitat preferences of nictating nematodes** Laura M. Kruitbos[1]; Stuart Heritage[2]; Mike J. Wilson[1], [1]University of Aberdeen, UK; [2]Forest Research, Roslin, Midlothian, UK

14:45 **162 STU** **Variability in desiccation tolerance among different strains of the entomopathogenic nematode *Heterorhabditis bacteriophora* Poinar** Mukuka John[1]; Strauch Olaf[1]; Ehlers U. Ralf[1], [1]Christian-Albrechts-Kiel University, Germany

15:00 **163 STU** **Analysis of the population development of *S. carpocapsae* and *S. feltiae* in liquid culture** Ayako Hirao[1]; Ralf Udo Ehlers[1], [1]University Kiel, Germany

15:15 **164** **Hunter to be hunted: Predator mites and entomopathogenic nematodes** Mehmet Karagoz[1]; Selcuk Hazir[2]; Ibrahim Cakmak[1]; Baris Gulcu[2]; Harry K. Kaya[3], [1]University of Adnan Menderes, Aydin, Turkey; [2]University of Adnan Menderes, Aydin, Turkey; [3]University of California, Davis, CA, USA

Contributed Papers Wednesday, 14:00-16:00. Arts C. Conf. Rm.

VIRUSES 5

Moderators: Lorena Passarelli and Nor Chejanovsky.

14:00 **165** **Baculovirus IE2 forms nuclear bodies in the nucleus and enhances CMV promoter expression in mammalian cells** Catherine Y. Y. Liu[1]; Chia-Hung Wang[1]; Wen-Kai Hsiao[1]; Yu-Chan Chao[1], [1]Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan, ROC

14:15 **166** **P35 is required for production of robust budded virus during AcMNPV infection of *Trichoplusia ni*** Bart Bryant[1]; Rollie J. Clem[1], [1]Kansas State University, Manhattan KS, USA

14:30 **167** **AcMNPV DNA replication is essential for P47 but not for LEF-4 expression** Mei Yu[1]; Eric B. Carstens[1],[1]Queen's University, Kingston, Canada

14:45 **168 STU** **Characterization of AcMNPV late expression factor 3 (LEF-3) functional domains for their role in nuclear localization and baculovirus DNA replication** Victoria Au[1]; Eric B. Carstens[1], [1]Queen's University, Kingston, Canada

15:00 **169 STU** **Removal of transposon target sites from AcMNPV *fp25k* delayed incidence of the FP phenotype but had no impact on DIP production in cell culture** Lopamudra Giri[1]; Huorang Li[2]; David Sandgren[2]; David W. Murhammer[1]; Bryony C. Bonning[2]; Mike Feiss[1]; Richard Roller[1], [1]University of Iowa, Iowa City, IA, USA; [2]Iowa State University, Ames, IA, USA

15:15 **170 STU** **Structural and functional analysis of the Chilo iridescent virus DNA polymerase promoter** Ikbal Agah Ince[1,2]; Remziye Nalçacıoğlu[2]; Zihni Demirbağ[2]; Just M. Vlak[1]; Monique M. van Oers[1],[1]Wageningen UR, The Netherlands; [2]Karadeniz Technical University, Trabzon, Turkey

15:30 **171 STU** **Suppression of AcMNPV gene expression in mammalian cells** Ryosuke Fujita[1]; Shinichiro Asano[1]; Ken Sahara[1]; Hisanori Bando[1], [1]Hokkaido University, Japan

15:45 **172** **SV40 polyadenylation (pA) signal increases transcription but reduces protein production in baculovirus expression vector system** Craig P. Seaborn[1]; Tamer Z. Salem[1]; Colin M. Turney[1]; Jianli Xue[1]; Xiao-Wen Cheng[1], [1]Miami University, Oxford, Ohio, USA

16:00–16:30 **BREAK** Arts Centre Gallery

Wednesday, 16:30-18:30. Arts Centre Gallery

POSTERS – 2

Posters should be displayed from Monday UNTIL NO LATER THAN 18:00 THURSDAY

MICROBIAL CONTROL

MC-00 **Production and evaluation of mosquitocidal efficacy of *Bacillus thuringiensis* subsp. *israelensis* based formulations in Vietnam** Binh D. Ngo[1]; Tuan D. Nguyen[1]; Ha T. Trinh[1], [1]Vietnamese Academy of Science and Technology, Hanoi, Vietnam

MC-01 **Comparison of phytopathogenic antagonism between *Bacillus subtilis* and *Bacillus thuringiensis* strains transformed with *chi*A gene from *Serratia marcescens* ATCC990** Feng-Chia Hsieh[1]; Jui-Tang Tseng[1]; Suey-Sheng Kao[1], [1]Taiwan Agricultural Chemicals and Toxic Substances Research Institute, Taiwan

MC-02 **Construction of a recombinant *Bacillus subtilis* strain as an integrated control agent being able to control to plant diseases and insect pests** Jong Yul Roh[1]; Jae Young Choi[1]; Yong Wang[1]; Hee Jin Shim[1]; Qin Liu[1]; Hong Guang Xu[1]; Jin-Cheol Kim[2]; Yeon Ho Je[1], [1]Seoul National University, Korea; [2]Korea Research Institute of Chemical Technology, Daejeon, Korea

MC-03 **Screening of *Bacillus thuringiensis* to the two-spotted spider mite *Tetranychus urticae*** Ricardo A. Polanczyk[1]; Dirceu Pratissoli[1]; Luiz Flávio V. Silveira[1]; Cláudio R. Franco[1]; Julieder G. Cocheto[1]; Launa P. de Souza[1]; Eduardo D. Grecco[1], [1]Centro de Ciências Agrárias - Universidade Federal do Espírito Santo, Brasil

MC-04 **Selection of *Bacillus thuringiensis* Cry toxins for the control of *Sitophilus oryzae* (Coleoptera: Curculionidae)** Najara da Silva[1]; Manoel Victor F. Lemos[1]; Ricardo A. Polanczyk[2]; Ana Maria G. Thuler[1]; Irlan L. de Abreu[1]; Camila C. Davolos[1]; Sergio B. Alves[3],[1]Universidade Estadual Júlio Mesquita Filho (UNESP Jaboticabal), Brasil; [2]Centro de Ciências Agrárias - Universidade Federal do Espírito Santo, Brasil; [3]Escola Superior deAgricultura Luis de Queiroz (ESALQ/USP), Brasil

MC-05 **Susceptibility of *Trichoplusia ni* (Lepidoptera: Noctuidae) to *Bacillus thuringiensis*** Ricardo A. Polanczyk[1]; Eduardo D. Grecco[1]; Dirceu Pratissoli[1]; Cláudio R. Franco[1]; Luiz Flávio V. Silveira[1], [1]Centro de Ciências Agrárias - Universidade Federal do Espírito Santo, Brasil

MC-06 STU **Effect of optical brighteners on the insecticidal activity of *Bacillus thuringiensis* ser. *kurstaki* and *Helicoverpa armigera* single nucleopolyhedrovirus** Maria A. Ibargutxi[1]; Alejandra Bernal[1]; Delia Muñoz[1]; Iñigo Ruiz de Escudero[1]; Primitivo Caballero[1], [1]Universidad Pública de Navarra, Spain

MC-07 **Future potential for biological control of *Neodiprion sertifer* Geoffr. and *Bupalus piniarius* L. in Latvia: occurrence and variability of pathogens** Liga Jankevica[1]; Rita Seskena[1]; Agnis Smits[2]; Ivars Zarins[1], [1]University of Latvia, Latvia; [2]Latvian State Forest Research Institute "Silava", Latvia

MC-08 **Searching for pathogens to control stored product mites (Acari: Acaridida)** Jan Hubert; Tomas Erban, Crop Research Institute, Prague, Czechia

MC-09 **Microbial control of insect pests in temperate orchard systems: Status and future prospects** David Shapiro-Ilan[1]; Lawrence A. Lacey[2], [1]USDA-ARS, Byron, GA, USA; [2]USDA-ARS, Wapato, WA, USA

MC-10 **Biological control of the fall webworm, *Hyphantria cunea* (Lepidoptera: Arctiidae) using a complex of entomopathogenic agents in Georgia** C. Chkhubianishvili[1]; I. Malania[1]; M. Kakhadze[1]; N. Mikaia[1], [1]Kanchaveli L. Institute of Plant Protection, Tbilisi, Georgia

MC-11 **Potential for entomopathogens against invasive species in landscape ornamentals in Florida** Steven P. Arthurs[1]; Lance Osborne[1], [1]Mid-Florida Research & Education Center, Apopka, FL, USA

MC-12 **Alkane-growth adaptation enhances virulence of *Beauveria bassiana* against *Triatoma infestans*, the major Chagas disease vector in Argentina** Nicolas Pedrini[1]; Carolina Cambiasso[1]; Patricia Juarez[1], [1]Instituto de Investigaciones Bioquímicas de La Plata (CCT CONICET-UNLP), Argentina

MC-13 **Effect of formulating of *Beauveria basssina* conidia on their viability and pathogenicity against the onion thrips, *Thrips tabaci*** Reyhaneh Ezzati-Tabrizi[1]; Reza Talaei-Hassanloui[1]; Aziz Kharazi-Pakdel[1]; Khalil Talebi[1], [1]University of Tehran, Karaj, Iran

MC-14 **Incidence, persistence and efficacy of *Beauveria bassiana* in cherry orchard soils** Joan Cossentine[1]; Paul Randall[1], [1]Pacific Agri-Food Research Centre, Summerland, BC, Canada

MC-15 STU **SEM study of the infection of the red palm weevil *Rhynchophorus ferrugineus* by *Beauveria bassiana*** Berenice Güerri-Agulló[1]; Sonia Gómez-Vidal[1]; Leticia Asensio[1]; Pablo Barranco[2]; Luis V. Lopez-Llorca[1], [1]Universidad de Alicante, Spain; [2]Universidad de Almería, Spain

MC-16 **Use of *Beauveria bassiana* as a tool for biological control of *Rhynchophorus ferrugineus*** Berenice Güerri-Agulló[1]; Leticia Asensio[1]; Pablo Barranco[2]; Sonia Gómez-Vidal[1]; Luis V. Lopez-Llorca[1], [1]Universidad de Alicante, Spain; [2]Universidad de Almería, Spain

MC-17 **Pathogenicity of *Beauveria bassiana* and *Cladosporium cladosporioides* to the two-spotted spider mite *Tetranychus urticae*** Ricardo A. Polanczyk[1]; Julieder G. Cocheto[1]; Dirceu Pratissoli[1]; Launa P. de Souza[1]; Luiz Flávio V. Silveira[1]; Cláudio R. Franco[1]; Sergio B. Alves[2], [1]Centro de Ciências Agrárias - Universidade Federal do Espírito Santo, Brasil; [2]Escola Superior de Agricultura Luiz de Queiroz (ESALQ/USP), Brasil

MC-18 STU **Occurrence and distribution of *Beauveria* and *Metarhizium* in Moroccan soil** Imoulan Abdessamad[1]; Alaoui Abdelaziz[1]; Elmeziane Abdellatif[1], [1]University Cadi-Ayyad, Marrakesh, Morocco

MC-19 **Evaluation of *Metarhizium anisopliae* for wireworm control in Switzerland** Ursula M. Kölliker[1]; Stefan Kuske[1]; Werner Jossi[1]; Christian Schweizer[1]; Siegfried Keller[1], [1]Agroscope Reckenholz-Tänikon Research Station ART, Zurich, Switzerland

MC-20 STU **Evaluating bioassay techniques for infection of *Rhipicephalus* ticks (Acari: Ixodidae) with entomopathogenic fungi** Felix Nchu[1]; Maniania M. Kalemba[1]; Ahmed Hassanali[1]; Kobus N. Eloff[2], [1]ICIPE - African Insect Science for Food and Health, Nairobi, Kenya; [2]University of Pretoria, South Africa

MC-21 **Evaluation on the potential of native fungal isolates against the Mexican bean bruchid, *Zabrutes subfaciatus* (Coleoptera: Bruchidae) in Ethiopia** Emiru S. Yeshanew[1]; Alemnesh H. Bedaso[1], [1]Addis Ababa University, Ethiopia

MICROSPORIDIA

M-01 ***Vairimorpha invictae* not detected in the parasitic fly, *Pseudacteon obtusus*, reared from the microsporidium-infected fire ants, *Solenopsis invicta*** David H. Oi[1]; Sanford D. Porter[1]; Steven M. Valles[1]; Juan A. Briano[2], [1]USDA-ARS Gainesville, FL, USA; [2]USDA-ARS Buenos Aires, Argentina

M-02 **A new *Cystosporogenes* isolate from *Agrilus anxius* (Coleoptera: Buprestidae)** George Kyei-Poku[1]; Debbie Gauthier[1]; Rian Schwarz[1]; Kees van Frankenhuyzen[1], [1]Great Lakes Forestry Centre, Canadian Forest Service, Sault Ste. Marie, Ontario, Canada

M-03 **Modeling horizontal transmission of microsporidia in *Lymantria dispar*** Dörte Goertz[1]; Gernot Hoch[1], [1]BOKU University of Natural Resources and Applied Life Sciences Vienna, Austria

NEMATODES

N-01 ***Hexamermis* sp. an entomopathogenous nematode associated with the European stink bug** Simona Landi[1]; Barbara Manachini[2], [1]El Colegio de la Frontera Sur (ECOSUR), Chiapas, México; [2]University of Palermo, Italy

N-02 **Habitat complexity effects on movement of *Steinernema carpocapsae* in maize** Randa Jabbour; Mary E. Barbercheck, Pennsylvania State University, University Park, PA, USA

N-03 **Pathogenicity of *Thripinema fuscum* Tipping & Nguyen (Tylenchida: Allantonematidae) infecting *Frankliniella fusca* (Hinds) (Thysanoptera: Thripidae)** Kelly R. Sims[1]; James J. Becnel[2]; Joseph E. Funderburk[3]; Drion G. Boucias[1], [1]University of Florida, Gainesville, FL, USA; [2]USDA/ARS, Gainesville, FL, USA; [3] North Florida Research and Education Center, University of Florida, Quincy, FL, USA

N-04 STU **Susceptibility of the Colorado Potato Beetle to the nematode *Pristionchus uniformis*** Andreas M. Weller[1]; Ralf J. Sommer[1], [1]Max Planck Institute for Developmental Biology, Tübingen, Germany

OTHER

O-01 **Toxicity of azadirachtin and some of its molecule analogue portions on larvae of *Galleria mellonella* (Lepidoptera) and on insect cell cultures** Carole Charbonneau[1]; Roland Côté[1]; Guy Charpentier[1], [1]Université du Québec à Trois-Rivières, Canada

O-02 **Cloning and expression of a venom protein from the endoparasitoid, *Pimpla hypochondriaca*, which has haemocyte anti-aggregation activity *in vitro*** M. P. Dani[1]; E. H. Richards[1], [1]Central Science Laboratory, Sand Hutton, York, UK

O-03 **A recombinant immunosuppressive protein from *Pimpla hypochondriaca* increases the susceptibility of two lepidopteran pests to *Bacillus thuringiensis*** E. H. Richards[1]; M. P. Dani[1], [1]Central Science Laboratory, Sand Hutton, York, UK

VIRUSES

V-01 **Characterization of white spot syndrome virus envelope protein VP51A and its interaction with viral tegument protein VP26** Yun-Shiang Chang[1]; Wang-Jing Liu[2]; Tsung-Lu Chou[1]; Yuan-Ting Lee[1]; Tai-Lin Lee[1]; Wei-Tung Huang[1]; Chu-Fang Lo[2], [1]Da-Yeh University, Taiwan; [2]National Taiwan University, Taipei, Taiwan

V-02 **Transactivation, dimerization, and DNA-binding activity of WSSV immediate early protein IE1** Wang-Jing Liu[1]; Yun-Shiang Chang[2]; Hao-Ching Wang[3]; Jiann-Horng Leu[1]; Guang-Hsiung Kou[1]; Chu-Fang Lo[1], [1]Institute of Zoology, National Taiwan University, Taiwan; [2]Da-Yeh University, Taiwan; [3]Institute of Biochemical Sciences, National Taiwan University, Taiwan

V-03 **Characterization of the *Amsacta moorei* entomopoxvirus spheroidin promoter** Srini C. Perera[1]; Peter J. Krell[2]; Basil M. Arif[1], [1]Great Lakes Forestry Centre, Canadian Forest Service, Sault Ste. Marie, Ontario, Canada; [2]University of Guelph, Guelph, Ontario, Canada

V-04 **Effects of chitinase J on the insecticidal efficacy of *Autographa californica* multiple nucleopolyhedrovirus** Tzyy-Rong Jinn[1]; Tsung-Lu Chou[1]; Suey-Sheng Kao[1], [1]Taiwan Agricultural Chemicals and Toxic Substances Research Institut, Taiwan, R.O.C.

V-05 **Reprogramming expression of chitinase and cathepsin of the *Autographa californica* multiple nucleopolyhedrovirus** Jeffrey J. Hodgson[1]; Noha Gerges[1]; Basil M. Arif[2]; Peter J. Krell[1], [1]University of Guelph, ON, Canada; [2]Great Lakes Forestry Centre, Sault Ste. Marie, ON, Canada

V-06 **Transactivation of *Epinotia aporema* granulovirus (EpapGV) promoters in *Anticarsia gemmatalis* cells** Marina Biedma[1]; Carolina Jaquenod De Giusti[1]; Alejandra Carrea[1]; Marcelo Berretta[1]; Alicia Sciocco-Cap[2]; Victor Romanowski[1], [1]Universidad Nacional de La Plata, Argentina; [2]Instituto Nacional de Tecnología Agropecuaria (INTA), Buenos Aires, Argentina

V-07 STU **Early gene *hhi1* of HzNV-1 virus is a strong apoptosis inducer and crucial for latent viral re-activation** Yueh-Lung Wu[1,2]; Song-Tay Lee[3];Yu-Chan Chao[1,2], [1]National Cheng Kung University, Taiwan ROC; [2]Academia Sinica, Taiwan ROC; [3]Southern Taiwan University of Technology, Taiwan ROC

V-08 **Functional analysis of two iap genes (iap2 and iap3) of *Lymantria xylina* multiple nucleopolyhedrovirus (LyxyMNPV)** Yu-Shin Nai[1]; Chung-Hsiung Wang[1], [1]National Taiwan University, Taiwan (R.O.C)

V-09 **Functional analysis of the putative antiapoptotic genes, *p49* and *iap4*, of *Spodoptera litura nucleopolyhedrovirus* with RNAi** Qian Yu[1]; Tiehao Lin[1]; Guozhong Feng[1]; Kai Yang[1]; Yi Pang[1], [1]Sun Yat-sen University, Guangzhou, China

V-10 STU **Anterograde trafficking of *Autographa californica* multiple nucleopolyhedrovirus is microtubule-dependent** John O. Danquah[1]; Ananya Jeshtadi[1]; Linda A. King[1], [1]Oxford Brookes University, Oxford, UK

V-11 STU **Structural analysis for cypovirus polyhedrin** Hanako Hibi[1]; Daisuke Nakai[1]; Norio Hamada[2]; Keiko Miura[3]; Peter Metcalf[4]; Hajime Mori[1], [1]Kyoto Institute of Technology, Japan; [2]Osaka University, Japan; [3]Japan

Synchrotron Radiation Research Institute, Hyogo, Japan; [4]University of Auckland, Auckland, New Zealand

V-12 Identification of viral factors required for the enhancer-like function of baculovirus polyhedrin upstream (*pu*) sequence Carol P. Wu[1]; Tou-Ya Huang[1]; Jen-Yeu Wang[1]; Huei-Ru Lo[1]; Yu-Chan Chao[1], [1]Academia Sinica, Nankang, Taipei, Taiwan, R.O.C.

V-13 Identification of putative miRNA sequences in four insect pathogenic viruses Woojin Kim[1]; John P. Burand[1], [1]University of Massachusetts - Amherst, Fernald Hall, Amherst MA, USA

V-14 MicroRNAs expressed in larval gypsy moth cells post parasitization by *Glyptapanteles flavicoxis* parasitoid Dawn Gundersen-Rindal[1], [1]USDA, Beltsville, MD, USA

V-15 Metagenomics of glassy-winged sharpshooter, *Homalodisca vitripennis* (Hemiptera: Cicadellidae) Wayne B. Hunter[1]; Kent S. Shelby[2]; Scot E. Dowd[3]; Catherine S. Katsar[4]; Phat M. Dang[5]; Laura E. Hunnicutt[6], [1]USDA, ARS, Ft. Pierce, FL, USA; [2]USDA, ARS, Columbia, MO, USA; [3]USDA, ARS, Lubbock, TX, USA; [4]USDA, APHIS-PPQ, Fort Lauderdale, FL, USA; [5]USDA, ARS, Dawson, GA, USA; [6]North Carolina State University, Raleigh, NC, USA

V-16 *Malacosoma neustria* nucleopolyhedrovirus (ManeNPV): Replication in Md203 cell line and host range in cell culture Remziye Nalcacioglu[1]; Nurten Gurel[1]; Ikbal Agah Ince[2]; Ismail Demir[1]; Zihni Demirbag[1],[1]Karadeniz Technical University, Trabzon, Turkey; [2]Giresun University, Turkey

V-17 STU The characteristics and viral susceptibility of the LD cloned cells, IPLB-LD-652Y cell strains-a~f Yi-Ting Yang[1]; Kuang-Hung Lin[1]; Wei-Fone Huang[1]; Chung-Hsiung Wang[1], [1]National Taiwan University, Taipei, Taiwan (R.O.C)

V-18 Applying an *Anticarsia gemmatalis* multiple nucleopolyhedrovirus (*Ag*MNPV)-based direct cloning system to make a cDNA expression library of the cottonwood borer beetle (*Plectrodera scalator*) Jeffrey M. Slack[1]; Olga Lihoradova[2]; Irina Ogay[3]; Shakhnoz Azimova[2]; John Dedes[1]; Rian Schwarz[1]; Peter J. Krell[4]; Basil M. Arif[1], [1]Great Lakes Forestry Centre, Sault STE Marie, ON, Canada; [2]Institute of Chemistry of Plant Substances, Tashkent, Uzbekistan; [3]Cambridge University , Cambridge, UK; [4]University of Guelph, ON, Canada

V-19 Optimization for high-throughput expression of recombinant protein using EasyBac system Jae Young Choi[1]; Yang-Su Kim[2]; Hee Jin Shim[2]; Yong Wang[2]; Jong Yul Roh[2]; Soo Dong Woo[3]; Byung Rae Jin[4]; Yeon Ho Je[2], [1]Seoul National University, Korea; [2]Seoul National University, Korea; [3]Chungbuk National University, Korea; [4]Dong-A University, Korea

V-20 Enhancement of recombinant proteins production in non-lytic insect cells expression system through simultaneously expression of baculovirus encoded transcriptional factor Chi-Hon Liao[1]; Yi-Ting Lin[1]; Tzong-Yuan Wu[1], [1]Department of Bioscience Technology, Chung Yuan Christian University, Chungli, Taiwan

V-21 STU Baculovirus as novel delivery tools for gene therapy in breast cancer Fernanda Murguia-Meca[1]; Richard B. Hitchman[2]; Linda A. King[1], [1]Oxford Brookes University, Oxford, UK; [2]Oxford Expression Technologies Ltd., Oxford Brookes University, Oxford, UK

V-22 Molecular cloning and characterization of a glycosyl hydrolase family 9 cellulase expressed throughout the digestive tract of the emma field cricket, *Teleogryllus emma* Namjung Kim[1]; Young-Moo Choo[2]; Kwang-Sik Lee[2]; Seong-Jin Hong[1]; Kwang-Youl Seol[1]; Byung-Rae Jin[2], [1]Department of Agricultural Biology, NIAST, Korea; [2]Dong-A University, Korea

V-23 Obtaining of recombinant human Müllerian Inhibiting Substance (MIS) by using baculovirus expression system Olga A. Lihoradova[1], Irina D. Ogay[1,2], Maria M. Podpisnova[1], Shakhnoz S. Azimova[1]; [1]The Academy of Sciences of Uzbekistan, Uzbekistan; [2]University of Cambridge, UK

V-24 STU Persistent infection and vertical transmission of *Spodoptera exigua* multiple nucleopolyhedrovirus (Hübner) (Lepidoptera: Noctuidae) Oihana Cabodebilla[1]; Oihane Simón[1]; Delia Muñoz[1]; Primitivo Caballero[1]; Trevor Williams[2], [1]Universidad Pública de Navarra, Spain; [2]Instituto de Ecología AC, Veracruz, Mexico

V-25 Hypermobility and climbing behaviour induced by baculovirus infection are regulated by separate gene functions Kelli Hoover[1]; Monique M. van Oers[2], [1]Pennsylvania State University, University Park, PA, USA; [2]Wageningen University, The Netherlands

V-26 Comparative pathology of the slow-killing *Adoxophyes honmai* NPV and *Autographa californica* MNPV in *A. honmai* Daigo Fujita[1]; Takayoshi Ishii[1]; Yasuhisa Kunimi[1]; Madoka Nakai[1], [1]Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan

V-27 Low oral infectivity of *Ac*MNPV in *Anticarsia gemmatalis* larvae correlates with hemocyte resistance to infection by budded virus Eric J. Haas-Stapleton[1]; Maggie Carrera[1]; Tiffany Chen[1]; Aniska Chikhalya[1]; Alisa de la Cruz[1]; Marianne Torres[1], [1]California State University, CA, USA

V-28 STU Investigations on the mechanism of CpGV resistance in *Cydia pomonella* Sabine Asser-Kaiser[1]; Gary Kaene[2]; Doreen Winstanley[2]; Johannes A. Jehle[1], [1]Agricultural Service Center Palatinate (DLR Rheinpfalz), Neustadt an der Weinstrasse, Germany; [2]Warwick Horticulture Research International, University of Warwick, Wellesbourne, Warwickshire, UK

V-29 Comparison of immune responses in *Cydia pomonella* granulovirus resistant and susceptible strains of *C. pomonella* Gary J. Keane[1]; Sabine Asser-Kaiser[2]; Marie Berling[3]; Miguel Ferber Lopez[3]; Johannes Jehle[2]; Doreen Winstanley[1], [1]Warwick HRI, University of Warwick, Wellesbourne, Warwickshire, UK; [2]Laboratory of Biotech. Crop Protection, Dept Phytopathology, DLR Rheinpfalz, Breitenberg, Germany; [3]EMA, Centre LGEI, Ales, France

V-30 Resistance of *Cydia pomonella* to granulovirus: Occurrence in Europe and tests on cross resistance with chemical insecticides Annegret Schmitt[1]; Benoit Sauphanor[2]; Johannes A. Jehle[3]; Juerg Huber[1], [1]JKI, Institute for Biological Control, Darmstadt, Germany; [2]National Institute of Agronomic Research, Avignon, France; [3]DLR Rheinpfalz, Laboratory for Biotechnological Crop Protection, Germany

V-31 Stability of resistance of codling moth against CpGV with and without virus pressure Karin Undorf-Spahn[1]; Eva Fritsch[1]; Juerg Huber[1], [1]JKI, Institute for Biological Control, Darmstadt, Germany

V-32 Comparative sequence analysis of two entomopoxviruses (EPVs) Zhen Li[1]; Christopher Lucarotti[2]; Peter J. Krell[3]; Basil M. Arif[1], [1]Great Lakes Forestry Centre, Sault Ste. Marie, Ontario, Canada; [2]Atlantic Forestry Centre, New Brunswick, Canada; [3]University of Guelph, Ontario, Canada

V-33 STU A new entomopoxvirus isolated from tea tortrix, *Homona coffearia*, in Sri Lanka Kiri Asano[1]; Keerthi Mohotti[2]; Yasuhisa Kunimi[1]; Madoka Nakai[1], [1]Tokyo University of Agriculture and Technology, Japan; [2]Tea Research Institute of Sri Lanka, Talawakelle, Sri Lanka

V-34 STU **Comparison between two new isolates of PhopGV from *Tecia solanivora* and *Phthorimaea operculella*** Carlos Espinel-Correal[1]; Xavier Léry[2]; Laura F. Villamizar[3]; Alba M. Cotes[3]; Miguel López-Ferber[1], [1]LGEI, Ecole des Mines, Alès, France; [2]IRD,Centre de Recherche, Alès, France; [3]CORPOICA-CBB, Cundinamarca, Colombia

V-35 STU **Determining the influence of transposon TCl4.7 insertion on the function of the genome of *Cydia pomonella* granulovirus** Wael H. El-Menofy[1,2] Johannes A. Jehle[1], [1]Agricultural Service Center Palatinate (DLR-Rheinpfalz), Germany; [2]Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Center (ARC), Egypt

V-36 **Quantitative PCR analysis of the tsetse fly salivary gland hypertrophy virus (SGHV) in a laboratory colony of *Glossina pallidipes*** Adly Abd-Alla[1]; François Cousserans[2]; Andrew G. Parker[1]; Alan S. Robinson[1]; Max Bergoin[2], [1]FAO/IAEA Agriculture & Biotechnology Laboratory, Agency's Laboratories, Vienna, Austria; [2]Université Montpellier II, France

V-37 **On the wings of Real Time: Detection, quantification, and effects of DWV** Aliya El Nagar[1]; Andrea Baker[1]; Matt Hall[1]; Declan Schroeder[1], [1]The Marine Biological Association, Citadel Hill, Plymouth, UK

18:30–19:30 **DINNER** Rootes Restaurant

SIP Division Business Meeting: Wednesday evening

Microbial Control (18:45-19:30) Arts Center Conf. Rm

Microbial Control Workshop Wednes., 19:30-21:30. Arts C. Conf Rm.

Biological Solutions to Pest Control

Organizer/Moderator: Kenneth Narva.

19:30 **173** **Challenges in commercialization of micro- and macro-biologicals** Andrew P. Brown[1], [1]Becker Underwood, Littlehampton, UK

19:45 **174** **Development of microbial biopesticides based on entomopathogenic fungi: Research to commercialization** Jarrod E. Leland[1], [1]Novozymes Biologicals, Salem, VA, USA

20:00 **175** **Field performance of novel stacked Bt products for protection against corn insects** Ken Narva[1]; Mike Culy[1]; Paul Neese[1]; Ed King[1]; Gary Thompson[1], [1]Dow AgroSciences, Indianapolis, IN, USA

176 Cancelled

20:15 **177** **Development of and prospects for the BtBooster platform technology** Milton D. Taylor[1]; Mohd Amir F. Abdullah[1]; Laura N. Frame[1]; Michael J. Adang[2], [1]Insectigen, Inc. Athens GA, USA; [2]The University of Georgia, Athens GA, USA

20:30 **178** **RNAi and Bt protein approaches to corn rootworm control** Thomas L. Clark[1], [1]Monsanto Company, Chesterfield, MO, USA

20:45 **179** ***Bacillus thuringiensis* - based products: Forever young** Dirk Ave[1], [1]Valent BioSciences, USA

21:30 **MIXER** Arts C. Theatre Bar

THURSDAY - 7 August

Symposium (Bacteria Division)Thursday, 8:00-10:00. Arts C. Theater

Commercialization and Quality Control of Bacterial Insecticides

Organizers/Moderators: Ralf-Udo Ehlers and Sergio Franceschini.

8:00 **180** **Bt standards and the importance of quality control of Bt products** Terry A. Benson[1], [1]Valent BioSciences Corporation, Long Grove IL, USA

8:30 **181** **Bacterial insecticides, commercial development and quality control** Changyan Chen[1], [1]Certis USA LLC, Columbia, MD, USA

9:00 **182** **Impact of regulations on commercialization of bacterial insecticides** Sergio Franceschini[1], [1]Intrachem Production, Grassobbio, Italy

9:30 **183** **Proposals for a balanced regulation of micobial biocontrol agents - results of the REBECA Action** Ralf-Udo Ehlers[1], [1]University of Kiel, Germany

Symposium (Virus Division) Thursday, 8:00-10:00. Arts C. Conf. Rm

Comparative Genomics of DNA Viruses

Organizer/Moderator: Elisabeth Herniou.

8:00 **184** **Evidence for extensive lateral acquisition of cellular genes by nucleocytoplasmic large DNA viruses** Jonathan Filée[1]; Michael Chandler[2], [1]LEGS / CNRS, Gif sur Yvette, France; [2]LMGM / CNRS, Toulouse, France

8:24 **185** **Mimivirus and Mimiviridae: Toward a new family of large DNA viruses** Jean-Michel Claverie[1]; Chantal Abergel[1], [1]CNRS-UPR Marseille, France (www.igs.cnrs-mrs.fr)

8:48 **186** **Structural divergence among genomes of closely related baculoviruses and its implications for baculovirus evolution** Robert L. Harrison[1], [1]USDA, ARS, Beltsville, MD, USA

9:12 **187** **The genome of *Oryctes rhinoceros* nudivirus: A missing link that solves some mysteries of invertebrate virus evolution** Yongjie Wang[1]; Monique van Oers[2]; Regina G. Kleespies[3]; M. B. Ramle[4]; Just M. Vlak[2]; Johannes A. Jehle[1], [1]DLR Rheinpfalz, Neustadt, Germany; [2]Wageningen University, The Netherlands; [3]Julius Kuehn Institute, Darmstadt, Germany; [4]Malaysian Palm Oil Board, Kuala Lumpur, Malaysia

9:36 **188** **Wasp-bracovirus associations: The grail quest for the ancestor virus** Annie Bézier[1]; Marc Annaheim[2]; Juline Herbinière[1]; Christoph Wetterwald[3]; Gabor Gyapay[4]; Sylvie Bernard-Samain[4]; Patrick Wincker[4]; Isabel Roditi[2]; Manfred Heller[2]; Maya Belghazi[5]; Jérôme Lesobre[1]; Rita Pfister-Wilhem[2]; Georges Periquet[1]; Catherine Dupuy[1]; Elisabeth Huguet[1]; Nathalie Volkoff[6]; Beatrice Lanzrein[2]; Jean-Michel Drezen[1], [1]IRBI CNRS, University of Tours, France; [2]University of Bern, Switzerland; [3]University of Bern, Switzerland; [4]Genoscope, Evry, France; [5]Faculté de Médecine Secteur Nord, Marseille, France; [6]BIVI INRA, Université de Montpellier II, France

Contributed Papers (Cross-Divisional) Thursday, 8:00-9:30. SS021

Pathogens of Bees

Moderator: Rosalind James.

8:00 **189** **A sticky situation: Picorna-like viruses infecting U.K. honeybee populations** Andrea C. Baker[1]; Aliya El Nagar[1]; Luke McKinder[1]; Matt J. Hall[1]; Declan C. Schroeder[1], [1]Marine Biological Association of the United Kingdom, Plymouth, UK

8:15 **190 STU** **Deformed wing virus in the parasitic mite, *Tropilaelaps* spp.** Eva Forsgren[1], Joachim R. de Miranda[1,2], Mats Isaksson[3], Shi Wei[4], Ingemar Fries[1], [1]Swedish University of Agricultural Sciences, Uppsala, Sweden, [2]Queen's University, Belfast, [3]National Veterinary Institute, Sweden, [4]CAAS, Beijing, China

8:30 **191 STU** **Honeybee immunity and parasitism by *Nosema* spp. fungi and *Varroa* mites** Catherine M. Little[1]; Dave Shutler[1], [1]Acadia University, Wolfville, NS, Canada

8:45 **192 STU** **Does fumagillin control the microsporidian *Nosema ceranae* in western honey bees (*Apis mellifera*)?** Geoffrey R. Williams[1]; Michelle A. Sampson[1]; Dave Shutler[1]; Richard E.L. Rogers[2], [1]Acadia University, Wolfville, Nova Scotia, Canada [2]Wildwood Labs Inc., Nova Scotia, Canada

9:00 **193 STU** **Environmental effects on fungal infections in honeybee larvae *Apis mellifera* (Hymenoptera: Apidae)** Svjetlana Vojvodic[1]; Annette Bruun Jensen[1]; Jørgen Eilenberg[1], [1]University of Copenhagen, Denmark

9:15 **194** **Asexual reproduction in the honey bee fungal pathogen *Ascosphaera apis*** Katherine A. Aronstein[1], Keith D. Murray[1,2], Robert A. Cramer[3], Thomas Eubanks[4], [1]USDA/ARS, Weslaco, TX, USA; [2]Weslaco, TX, USA; [3]Montana State University, Bozeman, MT, USA; [4]University of Texas-Pan American, Edinburg, TX, USA

10:00–10:30 **BREAK** BEB Lobby

Thursday, 10:30-12:30. Arts Centre Theatre

SOCIETY for INVERTEBRATE PATHOLOGY

Annual Business Meeting

Presiding: Wendy Gelernter

12:30–14:00 **LUNCH** Rootes Restaurant

Symposium (Cross-Divisional) Thursday, 14:00-16:00. Arts C. Theatre

Role of Disease in Regulation of Non-Pest Populations

Organizers/Moderators: Helen Roy, Judith Pell and John Burand.

14:00 **195** **Specialist and generalist entomopathogenic fungi infecting non-pest insects: Implications for ecosystem services and relevance of behavioural ecology** Nicolai V. Meyling[1]; Jørgen Eilenberg[1], [1]University of Copenhagen, Denmark

14:24 **196** **Covert viruses in wild populations** Rosie S. Hails[1], [1]NERC Centre for Ecology and Hydrology, Oxford, UK

14:48 **197** **Microsporidian disease in beneficial insects** Leellen F. Solter[1], [1]Illinois Natural History Survey, Illinois, USA

15:12 **198** **Methods for studying pathogens in natural populations: Recent developments and future thoughts** Helen Hesketh[1], NERC CEH, Mansfield Road, Oxford, UK

15:36 **199** **Parasites mediate biological invasions** Alison M. Dunn[1], [1]Biological Sciences, University of Leeds, UK

Contributed Papers Thursday, 14:00-15:30. Arts C. Theatre

BACTERIA 4

Moderator: Hyun-Woo Park.

14:00 **200** **Genetic improvement of the Cry11 from *Bacillus thuringiensis* subsp. *medellin* by directed molecular evolution** Alvaro M. Flórez[1]; Gloria M. Morales[1]; Sergio Orduz[2], [1]Universidad de Santander, Bucaramanga, Colombia; [2]Universidad Nacional de Colombia sede Medellin and Corporación para Investigaciones Biológicas, Medellín, Colombia

14:15 **201** **Characteristics of a *sigL* mutant in *Bacillus thuringiensis* HD-73** Qi Peng[1,2]; Li Zhu[1]; Fuping Song[1]; Jie Zhang[1]; Jiguo Gao[2]; Dafang Huang[1], [1]Chinese Academy of Agricultural Sciences, Beijing, China; [2]Northeast Agricultural University, Harbin, China

14:30 **202** **The characteristics of an antagonistic *Bacillus thuringiensis* strain against crop pathogens and pests** Miao M. Hang[1]; Liang Xiao[1]; Jun Cai[1,2]; Chi C. Xie[1]; Yuehua Chen[1,2], [1]Nankai University, P.R.China; [2]Ministry of Education, P.R.China

14:45 **203** **Characterization of mosquitocidal *Bacillus cereus* toxic to *Ochlerotatus taeniorhynchus* and *Culex quinquefasciatus*** Hyun-Woo Park[1]; Sabrina R. Hayes[1], [1]Florida A & M University, Panama City, FL, USA

15:00 **204** **Pathogenesis of male-killing *Wolbachia* in *Drosophila bifasciata*** Aurore Dubuffet[1], Zoe Veneti[2], Henk R. Braig[3], Judith E. Smith[1], Greg D. D. Hurst[2], [1]University of Leeds, UK; [2]University of Liverpool, UK; [3]University of Wales, UK

15:15 **205** ***Brevibacillus laterosporus* potential against the house fly and its safety for the non-target pupal parasitoid *Muscidifurax raptor*** Luca Ruiu[1]; Alberto Satta[1]; Ignazio Floris[1]; David J. Ellar[2], [1]University of Sassari, Italy; [2]University of Cambridge, UK

Contributed Papers Thursday, 14:00-15:45. SS021

MICROBIAL CONTROL 3

Moderator: Caroline Hauxwell.

14:00 **206** **Toward aphid-resistant transgenic plants** Sijun Liu[1]; Zhaouhui Wang[2]; S. Sivakumar[1]; Liljana Georgievska[1]; Glenn F. King[3]; W. Allen Miller[2]; Bryony C. Bonning[1], [1,2]Iowa State University, Ames, IA, USA; [3]Institute for Molecular Bioscience, Brisbane, Australia

14:15 **207** ***Yersinia* n. sp. EN65 a novel insecticidal bacterium: A new biocontrol agent for diamondback moth, *Plutella xylostella*?** Michael Brownbridge[1]; Mark R.H. Hurst[1], [1]AgResearch Limited, New Zealand

14:30 **208** **Biochemical characterization and insecticidal activity of an alkaline metalloprotease produced by *Photorhabdus luminescens* 0805-P5G isolated from Taiwan** Feng-Chia Hsieh[1]; Yu-Tzu Chang[2]; Suey-Sheng Kao[1], [1]Taiwan Agricultural Chemicals and Toxic Substances Research Institute, Council of Agriculture, Taiwan; [2]Asia University, Taiwan

14:45 **209** **Heterologous expression of recombinant bacterial endochitinases and production of chitin-derived oligosaccharides** J. Eleazar Barboza-Corona[1]; O. B. Gutierrez-Acosta[1]; M. Imperial-Cervantes[1]; Dennis K. Bideshi[3,4]; N. de la Fuente-Salcido[1,2]; R. Salcedo-Hernandez[1], [1]Universidad de Guanajuato, Guanajuato, Mexico; [2]Universidad Autonoma de Coahuila, Mexico; [3]California Baptist University, Riverside, California, USA; [4]University of California, Riverside, USA

15:00 **210** **Plusiine baculoviruses: Potential for cabbage looper, *Trichoplusia ni*, control in greenhouse vegetable production** Martin A. Erlandson[1]; Dave Gillespie[2]; David Theilmann[3] [1]Agriculture and Agri-Food Canada, Saskatoon Research Centre, SK, Canada; [2]Agriculture and Agri-Food Canada, Agassiz, BC, Canada; [3]Agriculture and Agri-Food Canada, Pacific Agriculture Research Centre, Summerland, BC Canada

15:15 **211** **Use of a granulovirus (PoGV) and *Bacillus thuringiensis* (Bt) to control potato tuber moth (*Phthorimaea operculell*)** Steven P. Arthurs[1]; Lawrence A. Lacey[1], [1]USDA-ARS, Wapato, WA, USA

212 Cancelled

15:30 **213** **Finding a microbial control agent for the invasive crayfish, *Orconectes virilis*** Elizabeth W. Davidson[1], Jennifer L. Snyder[2], Donald Lightner[3], Marcia Kyle[1], [1]Arizona State University, Tempe AZ, USA; [2]University of Arizona Agriculture Center, Maricopa, AZ, USA; [3]Veterinary Science/Microbiology, University of Arizona, Tucson, AZ, USA

16:00–16:30 **BREAK** Arts Centre Gallery

Symposium (Microbial Control) Thursday, 16:30-18:30. Arts C. Theatre

Regulatory and Market Barriers for Approval of Microbial Control Products

Organizer/Moderator: David Chandler.

16:30 **215** **Regulatory innovation and biopesticide commercialization** Wyn P. Grant[1]; Justin G. Greaves[1]; David Chandler[2]; Gillian Davidson[3]; G Mark Tatchell[3], [1,3]University of Warwick , Coventry, UK; [2]Warwick HRI, University of Warwick, Wellesbourne, UK

17:00 **216** **Microbial control products: The regulatory challenge** John Dale, Pesticides Safety Directorate, York, UK

17:30 **217** **Commercialization of microbial control products: The industry perspective** Dirk Ave[1], [1]Valent BioSciences, USA

18:00 **218** **Understanding the adoption of alternative pest management strategies: An economist's view** Alastair Bailey, University of Kent, Canterbury, Kent, UK

Contributed Papers Thursday, 16:30-18:30. SS021

BACTERIA 5

Moderator: Samir Naimov.

16:30 **219** ***B.t.*-toxins in the midgut of Western corn rootworm (*Diabrotica virgifera virgifera* LeConte)** Renate Kaiser-Alexnat, Julius Kuehn Institute, Darmstadt, Germany

16:45 **220** **Mutations in the *cadherin* gene in a *O. nubilalis* strain selected for Cry1Ab resistance** Yolanda Bel[1]; Blair D. Siegfried[2]; Juan Ferré[1]; Baltasar Escriche[1], [1]University of Valencia, Spain; [2]University of Nebraska, Lincoln, NE, USA

17:00 **221** ***Bacillus thuringiensis* Cry2A toxins bind saturably to a common site in the midgut of *Helicoverpa armigera*** C. Sara Hernández-Rodríguez[1]; Adri Van Vliet[2]; Nadine Bautsoens[2]; Jeroen Van Rie[2]; Juan Ferré[1], [1]Universitat de València, Spain; [2]Bayer BioScience N.V., Gent, Belgium

17:15 **222** **The importance of antibiosis and inter-specific competition in the ecology of *Bacillus thuringiensis*** Ben Raymond[1]; Michael B. Bonsall[1], [1]Oxford University, UK

17:30 **223** **REPAT proteins and their role in the tolerance of *Spodoptera exigua* to its pathogens** Carmen S. Hernandez[1]; Patricia Hernandez-Martinez[1]; Gloria Navarro-Cerrillo[1]; Juan Ferre[1]; Baltasar Escriche[1]; William J. Moar[2]; Ruud A. de Maagd[3]; Salvador Herrero[1], [1]Universitat de Valencia, Spain; [2]Auburn University, Auburn, AL, USA; [3]Plant Research International B.V., Wageningen, The Netherlands

17:45 **224** **Cloning and expression of the Cry1Ac-binding alkaline phosphatase (HvALP) from *Heliothis virescens*** Omaththage P. Perera[1]; Jonathan D. Willis[2]; Michael J. Adang[3]; Juan Luis Jurat-Fuentes[2], [1]USDA-ARS Stoneville, MS, USA; [2]University of Tennessee, Knoxville, TN, USA; [3]University of Georgia, Athens, GA, USA

18:00 **225** **Cloning of a Cry3Aa-receptor cadherin from *Tenebrio molitor*** Jeff Fabrick[1]; Cris Oppert[2]; Marcé Lorenzen[3]; Brenda Oppert[3]; Juan Luis Jurat-Fuentes[2], [1]USDA-ARS Maricopa, AZ USA; [2]University of Tennessee, Knoxville, TN, USA; [3]USDA-ARS Manhattan, KS, USA

18:15 **226** ***Bacillus thuringiensis* camelysin accumulates in biofilm and is also in vivo expressed** Thomas Candela; Christophe Buisson; Nathalie Gilois; Stéphane Aymerich; Didier Lereclus; Christina Nielsen-LeRoux; Michel Gohar, INRA, France

Contributed Papers Thursday, 16:30-18:15. Arts C. Conf. Rm.

VIRUSES 6

Moderators: Monique van Oers and Adly Abd-Alla.

16:30 **227** **"Here's spitting at you, kid" - Oral transmission of the *Musca domestica* salivary gland hypertrophy virus (MdSGHV) via salivary secretions** Verena U. Lietze[1]; Christopher C. Geden[2]; Drion G. Boucias[1], [1]University of Florida, Gainesville, FL, USA; [2]USDA-ARS, Gainesville, FL, USA

16:45 **228** **MdSGHV transcriptome during viral infection in the house fly** Tamer Z. Salem[1,2]; James E. Maruniak[1]; Verena U. Lietze[1], Drion G. Boucias[1], [1]University of Florida, Gainesville, Florida, USA; [2]AGERI, Agricultural Research Center, Egypt

17:00 **229** **Isolation and functional analysis of an ascovirus-encoded microRNA regulating viral replication** Mazhar Hussain; Ryan J. Taft; Sassan Asgari, University of Queensland, St Lucia, Australia

17:15 **230** **Immobilization of proteins into *Bombyx mori* cypovirus polyhedra** Hajime Mori[1]; Hiroshi Ijiri[1]; Gento Nishimura[1]; Takeshi Nakatani[1]; Keiko Ikeda[2]; Fasseli Coulibaly[3]; Elaine Chiu[3]; Peter Metcalf[3], [1]Kyoto Institute of Technology, Japan; [2]Protein Crystal Corporation, Osaka, Japan; [3]University of Auckland, New Zealand

17:30 **231** **Flies infected with *Wolbachia* are less susceptible to *Drosophila C virus*** Karyn N. Johnson[1]; Jeremy C. Brownlie[1]; Lauren M. Hedges[1], [1]University of Queensland, Brisbane, Australia

17:45 **232** **Pathological effects and possible ecological impact of newly identified viruses of the aphids *Brevicoryne brassicae* and *Dysaphis plantaginea*** Eugene V. Ryabov[1]; Gary Keane[1]; Neil Naish[1]; Doreen Winstanley[1], [1]University of Warwick, Warwick HRI, Wellesbourne, Warwick, UK

18:00 **233** **Positive-strand RNA viral infections of the red imported fire ant, *Solenopsis invicta*** Steven M. Valles, USDA-ARS, Gainesville, FL, USA

IMPORTANT NOTE: Remove all posters before 18:00

19:00 **BANQUET** & **AWARDS CEREMONY** Britannia Royal Court Hotel

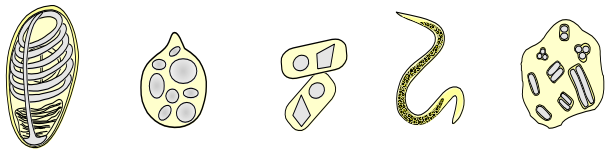
19:00-20:00 **Cocktail hour**

20:00 **Banquet**

"Safe may'st thou wander, safe return again!"

Cymbeline, Act III, Scene V

We hope to see you in Utah for SIP 2009 !



ABSTRACTS 2008

IMPORTANT NOTES:

These abstracts should not be considered to be publications and should not be cited in print without the author's permission.

STU indicates papers being judged for graduate student presentation awards

129 indicates abstract number for ORAL presentation

B-11 indicates abstract number for POSTER presentation

MONDAY - 4 August

PLENARY SYMPOSIUM Monday, 10:30–12:30

Honey Bee Colony Collapse Disorder

PLENARY SESSION. Monday, 10:30. **1**

Colony Collapse Disorder (CCD): CSI in the bee hive

Dennis vanEngelsdorp[1]
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In North America, populations of the honey bee *Apis mellifera* have been in decline since the introduction of the varroa mite, *Varroa destructor*, in the 1980's. Parasitization by varroa mites still is a major factor underlying most colony losses, most likely through immunosuppression and increased disease instance. However, a new phenomenon was identified in late 2006 that is though to be responsible for large colony losses in affected apiaries: colony collapse disorder (CCD). This condition is identified by a set of unique symptoms: no dead bees in the affected hive or apiary, honey bee brood and food stores are left behind, and secondary pests hesitate to invade affected hive equipment. CCD has continued to have major impact on bee colonies in the United States and significantly add to the already high loss of colonies due to varroa parasitization. In an attempt to determine the cause or causes of CCD, several studies were initiated. Common samples were collected from CCD and non-CCD affected apiaries and shared among various institutions in an attempt to isolate a single causes. No one culprit has yet been found which explain all CCD losses. A longitudinal epidemiological study was also initiated in 2007 that followed individual colonies over time, sampling them repeatedly. This study uncovered several factors which impact bee health but not necessarily how CCD is triggered. This presentation will discuss the approaches being taken to investigate causes of colony losses, and how losses in the United States compares to losses in other countries in terms of magnitude, symptoms and response.

PLENARY SESSION. Monday, 11:00. **2**

Microsporidia infections in hymenopteran pollinators

Ingemar Fries[1]
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Phylogenetically, Microsporidia are now considered highly specialised parasitic fungi. They are all intracellular parasites with a characteristic and unique mode of infection. Microsporidia may infect all life forms and undoubtedly, only a small fraction of the actual number of species have been characterised. In Hymenopteran pollinators, microsporidia infections have been described from four host species only: *Nosema apis* infecting the European honey bee, *Apis mellifera; Nosema ceranae* infecting the Asian honey bee, *Apis cerana; Nosema bombi*, infecting *Bombus* spp. and *Antonospora scoticae* infecting *Andrena scoticae. N. apis* and *N. ceranae* are cross infective between hosts. However, *N. apis* does not do well in *A. cerana*, whereas there is a worldwide process of *N. ceranae* replacing *N. apis* in *A. mellifera*. *N. bombi* has recently become of particular interest for conservationists, since this parasite may be distributed to areas assumed free from this parasite, thereby presumably endangering endemic bumble bee spp. Furthermore, within-genome rRNA variability in *N. bombi* suggests that to characterize intraspecific genetic variants in the Microsporidia based on RNA sequences is not straight forward. *A. scoticae* infects the fat body tissue of *A. scotica* and may occur with an extreme prevalence in its host.

PLENARY SESSION. Monday, 11:30. **3**

Applied beenomics: Molecular studies of honey bee disease and resistance

Jay D. Evans[1]
[1]USDA, ARS, Beltsville, MD, USA.
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Honey bee colonies face disease threats ranging from viruses to bacteria and mites. Recent severe colony losses in North American honey bees reflect, in part, a rare syndrome during which adult bees disappear from colonies, leaving behind healthy queens and brood with no obvious pathologies. Current hypotheses to explain this syndrome, Colony Collapse Disorder, center on nutritional deficiencies in bees, exposure to harmful exogenous chemicals, and the presence of new or resurgent pathogens. These hypotheses have been tested by genetic analyses of honey bee pathogens and gene-expression analyses of honey bee genes involved in immunity and stress responses. Copy numbers of several bee viruses as well as trypanosomatid parasites are positively correlated with CCD, with a substantial geographic component to the predominant pathogens. Several honey bee genes have emerged as expression biomarkers for CCD, although CCD and control bees do not show systematic differences in the expression of genes related to immune function or stress responses. Genomic resources for honey bees and their major pathogens are also being used to improve honey bee breeding and management for disease resistance. Heritability and efficacy of immune genes targeting the bacterial pathogen *Paenibacillus larva* will be discussed, along with efforts to use molecular tools to follow bee-bacterium interactions.

PLENARY SESSION. Monday, 12:00. **4**

Unraveling the pathogens in honey bees undergoing Colony Collapse Disorder

Diana Cox-Foster[1]
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Colony Collapse Disorder (CCD) was first recognized in 2006 in several beekeeping operations and presented symptoms previously not known. Collapse of the colony occurred rapidly over a short time, with loss of most workers leaving behind the queen, a small number of newly emerged workers, and brood. Analysis of remaining bees revealed large numbers of known pathogens in individual bees, without any one pathogen being linked to the symptoms. This presentation will discuss the collaborative efforts to identify pathogens involved in CCD using a metagenomic analysis of bees taken colonies having identified CCD versus historical and healthy colonies. This analysis resulted in the identification of four pathogens that were strongly linked to CCD, the Israeli Acute Paralysis Virus, the Kashmir Bee Virus, Nosema ceranae, and Nosema apis. Further examination of IAPV reveals that multiple lineages exist, with at least two being present in CCD colonies in the United States and Canada. Additional studies will be described in which colonies were exposed to IAPV in containment greenhouses and symptoms observed. Current questions will be discussed concerning the role of pathogens in Colony Collapse Disorder and bee health worldwide.

SYMPOSIUM (Cross Divisional) Monday, 14:00-16:00

Invertebrate Pathogens as Models for Basic Ecological and Evolutionary Principles

Symposium. Monday, 14:00. **5**

Where theory meets reality: Viral disease in field populations of forest Lepidoptera

Jenny Cory[1]; Judy Myers[2]
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[2]Departments of Zoology and Agroecology, University of British Columbia, 6270 University Boulevard, Vancouver, BC, V6T 1Z4, Canada.
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Viral disease is a major component of the cyclic population dynamics of some Lepidoptera including western tent caterpillars. Epizootics of nucleopolyhedrovirus and host population subdivision provide an arena in which selection on virulence of virus and resistance of hosts could act. Theory predicts that epizootics should select for host resistance and that viral isolates should respond to this change on a population-by-population basis. Experiments provide evidence that these interactions are occurring but that patterns are weak as compared to other factors that determine the cyclic population dynamics. In addition there is no evidence for induced immunity or selection within a generation of tent caterpillars. The factors that promote the rapid development of NPV epizootics remain a mystery and are the topic of future research.

Symposium. Monday, 14:30. **6**

Baculoviruses as a model of host shifts and disease emergence

Amy B. Pedersen[1] [1]University of Sheffield, UK.
Address for correspondence: a.pedersen@sheffield.ac.uk

Many recent emerging infectious diseases in humans, such as HIV and Ebola resulted from host shifts, are maintained within wildlife populations, and pose a substantial health risk. Most research has focused on controlling epidemics, however, using model systems can formulate predictions about the factors that lead to successful disease emergence. Here, I employ an insect-virus system to test the conditions that lead transient infections to become self-sustaining diseases. The Indian meal moth (Plodia interpunctella) and the Almond moth (Ephestia cautella) are worldwide pests of stored food products, and due to their tractability in laboratory experiments have been used to study host-parasite dynamics. EcNPV, a nucleopolyhedrovirus, is largely host specific on Ephestia, but can be transmitted to the new host, Plodia; demonstrating altered disease expression. PiGV, a granulosis virus, is host specific on Plodia, with little evidence of transmission to Ephestia. Here, I measure how infection route affects the infectivity of each virus on both hosts; testing the standard oral inoculation route versus direct intrahaemocoelic injections of the inclusion-bodied virus on important epidemiological parameters (infectivity, disease induced mortality, sub-lethal effects, covert infection). These findings will elucidate the important components of host-pathogen dynamics that can lead to long-term sustainability of emerging diseases.

Symposium. Monday, 15:00. **7**

Host-parasite coevolution under environmental variation

Tom J. Little[1] [1]University of Edinburgh, UK.
Address for correspondence: tom.little@ed.ac.uk

Invertebrate and other animal populations harbour genetic variation for immune capacity, which may seem paradoxical given the importance of immune performance to fitness. Why is functional variation in immune capacity not purged by natural selection? Why are susceptibility alleles not eliminated? Accumulating evidence suggests that environmental heterogeneity may retard the long term efficiency of natural selection and even maintain polymorphism, provided alternate host genotypes are favoured under different environmental conditions. “Environment” in this context may refer to abiotic factors such as temperature or food availability, or the genetic diversity of pathogens. These factors are controlled in many laboratory experiments measuring pathogen resistance, and yet they may be overwhelmingly important in the evolution of resistance, virulence, and, ultimately, coevolution. In this talk, I will discuss how the abiotic environment interacts with host and parasite genotypes to shape the evolutionary interactions between the crustacean Daphnia magna and its bacterial parasite Pasteuria ramosa.

Symposium. Monday, 15:30. **8**

The evolutionary ecology of Bt

Michael B. Bonsall[1] [1]Oxford University, UK.
Address for correspondence: michael.bonsall@zoo.ox.ac.uk

The factors affecting the evolutionary ecology and dynamics of the interaction of Bt (*Bacillus thuringiensis*) with its lepidopteran host, the diamondback moth (DBM) will be discussed. The evolution of host resistance to Bt threatens the sustainable use of this bacteria to modern agriculture and our understanding of this host-pathogen interaction provides a fantastic system in which to explore ideas about the evolutionary ecology of pathogen virulence, pathogen transmission and host resistance. We will focus on three aspects of our research from within-host to field dynamics. First, from a detailed study of the within-host mechanisms of infection, we will discuss how the presence of alternative (non-toxin) genes (or Bt-related bacteria that express these non-toxin genes) are essential to Bt infectivity and transmission. Second, selection experiments have revealed how DBM resistance evolves in relation to Bt strain diversity and host population density and this work will be considered in conjunction with evolutionary theory on pathogen virulence and host resistance. Finally, from field experiments, we will illustrate how the diversity and population structure of native Bt floras (and related bacteria) are affected by the presence of pest insects (e.g., DBM) and/or Bt-based insecticides (e.g., DiPel).

CONTRIBUTED PAPERS Monday, 14:00-15:45

FUNGI 1

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

The fascinating true story about the famous *Metarhizium anisopliae* isolate Ma43, alias ATCC 90448, alias BIPESCO 5, alias F52 alias …….

Jørgen Eilenberg[1]; Gisbert Zimmermann[2]; Tariq Butt[3]; Kerstin Jung [2]; Charlotte Nielsen[1]; Hermann Strasser[4]; Milton Typas[5]
[1]Department of Ecology, University of Copenhagen, Thorvaldsensvej 40, DK 1871 Frb. C., Denmark, [2]BBA, Institute for Biological Control, Heinrichstrasse 243, D-64287 Darmstadt, Germany, [3]Department of Biological Sciences, University of Swansea, Singleton Park, Swansea, Wales, UK SA2 8PP, UK, [4]Institute of Microbiology, University of Innsbruck, Technikerstrasse 25, A-6020 Innsbruck, Austria, [5]University of Athens, Department of Genetics and Biotechnology, GR-TR15701, Athens, Greece.
Address for correspondence: Jei@life.ku.dk

In 1971, dead larvae of the codling moth, *Cydia pomonella*, were send from Austria to The Institute for Biological Control (BBA) in Darmstadt, Germany, for diagnosis of diseases. From one larva, *Metarhizium anisopliae* was isolated and given the name Ma43 in the local culture collection. The isolate proved to be pathogenic to a range of insects and it was send to different laboratories. In addition, it became the basis of commercial development of *M. anisopliae* BIO 1020 for biological control of the black vine weevil. Later, the isolate became the active ingredient of several other commercial products in the USA. Over time, descendants of this isolate were given many names by different laboratories and culture collections and were used in many laboratory and field studies. Thus, literature studies based on descendants can refer to the fungus as Ma43, ATCC 90448, BIPESCO 5, F52, 275-86, KVL 99-112 and others. This raises a range of questions: Should all published studies basically be regarded as referring to the same isolate? What are the consequences if different descendants show different genetic profile and/or different biological properties? Can we extract some general guidelines?

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

A novel approach to develop biopesticides based on entomopathogenic fungi

Kim . Jae Su[1]; Woo Eun Ok[1]; Park Jong Sung[1]; Kim Yun Sung[1]; Kim Tae-Joon[1]; Kim Kyoung-Sung[1]; Roh Jong Yul[2]; Choi Jae Young[2]; Je Yeon Ho[2.]
[1]AgroLife Research Institute, Dongbu HiTek Co. Ltd., Daejon 305-708, Korea, [2]School of Agricultural Biotechnology, College of Agriculture & Life Science, Seoul National University, Seoul 151-742, Korea.
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Among the cultured products of *Beauveria bassiana* SFB-205 (KCCM 10892P), the supernatant showed the highest insecticidal activity against 2nd instars of *Aphis gossypii* (Aphididae) nymphs under glasshouse conditions. The enzymes in the supernatant were confirmed as active materials, and the chitinase was finally selected as a QC factor for commercial production. However, the chitinase activity in the supernatant decreased by 11-folds due to the thermal stress at 50°C for 2 h. To improve this thermal unstability, the chitinase in the supernatant was adsorbed to a precipitable material, and the pellet was freeze-dried after centrifugation (PCT/KR2007/005886). The selected adsorbent showed the highest harvesting efficiency of 92.7%. The chitinase activity of the freeze-dried powder was maintained up to 82.0% of initial activity under the same thermal stress condition. Among the recipes tested, the oil-based formulation was stable up to 18 months at room temperature and resulted in 96.1% control efficacy against 2nd instars of *A. gossypii* nymphs 1 day after the treatment. This approach could be a practical method to develop biopesticides including active metabolites from the entomopathogenic fungi. Further study for improving the product quality is underway.

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

Host plant effects on fitness of the mite pathogenic fungus *Neozygites floridana*

Vitalis W. Wekesa[1]; Stefania Vital[1]; Renan A. Silva[1]; Italo Delalibera Jr.[1]
[1]Department of Entomology, Plant Pathology and Agricultural Zoology, University of São Paulo (USP-ESALQ Campus), ESALQ-USP, C.P. 9 13418–900, Piracicaba, São Paulo, Brazil.
Address for correspondence: ewekesah@yahoo.com

Host plant effects on two isolates of *Neozygites floridana* Weiser & Muma to the spider mites *Tetranychus evansi* Baker & Pritchard and *Tetranychus urticae* Koch was investigated. Percent mortality, mummification and sporulation after host death was evaluated for *T. evansi* reared on tomato, cherry tomato, eggplant, nightshade, and pepper while *T. urticae* was reared on strawberry, jack bean, cotton and *Gerbera*. Mite fecundity was determined on each plant to infer host plant suitability. The effect of host plant on contamination, infection, mummification and sporulation of *N. floridana* isolate pathogenic to *T. urticae* was very small. On the other hand, all parameters of the isolate pathogenic to *T. evansi* were significantly affected by the host plants. For example, mummification of *T. evansi* reared on tomato was 3 times higher than nightshade. Oviposition was positively correlated to the measured fungal parameters on all host plants with the exception of nightshade and pepper. On nightshade, although oviposition (30 eggs/female) and infection (81.3%) were high, only 24.7% of the infected mites became mummified. Oviposition of *T. evansi* on pepper was also very low (5 eggs/female) and although infection/mummification was relatively high, sporulation was the lowest among all host plants, suggesting that antibiosis may affect both mite reproduction and fungal activity.

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

Intraguild interactions involving *Pandora neoaphidis* at the population scale

Jason Baverstock[1]; Judith K. Pell[1]
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Laboratory scale experiments have demonstrated intraguild interactions between the aphid-specific entomopathogenic fungus *Pandora neoaphidis*, the coccinellid *Coccinella septempunctata* and the aphid parasitoid *Aphidius ervi*. These interactions can have positive (enhanced transmission) or negative (intraguild predation) effects on the fungus. Whereas the intraguild interactions between *P. neoaphidis* and *C. septempunctata* are unidirectional, with the fungus having no direct effect on the coccinellid, the interactions between *P. neoaphidis* and *A. ervi* can be bidirectional. Previous experiments that have assessed the interactions within this guild were done within highly artificial arenas under abiotic conditions that were optimal for the fungus. The use of mesocosms allows intraguild interactions to be assessed at the population scale under abiotic conditions similar to that of the field. Here we describe experiments in mesocosms ranging in size from insectary cages to glasshouses. Experiments which assessed the competitive interactions between guild members at the population scale will be described along with those done to assess the effect of plant diversity on the co-existence of aphid natural enemies.

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

Enhanced transmission of *Pandora neoaphidis* by the invasive ladybird *Harmonia axyridis*

Patricia M. Wells[1]; Jason Baverstock[1]; Michael E.N. Majerus[2]; Helen E. Roy[3]; Judith K. Pell[1]
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Harmonia axyridis is an invasive ladybird native to Asia that has established in the UK and is an intraguild predator of the aphid specific pathogenic fungus *Pandora neoaphidis*. The native predator *Coccinella septempunctata* partially consumes aphids infected with the fungus, however, enhanced transmission and vectoring of the fungus in the presence of the predator reduces the impact of intraguild predation. In contrast *H. axyridis* entirely consumes *P. neoaphidis* sporulating cadavers. Here we assessed the effect of *H. axyridis* on the transmission of *P. neoaphidis* on single plants relative to the effect of *C. septempunctata*. *Harmonia axyridis* is comparable to *C. septempunctata* in both enhancing within and between (vectoring) plant transmission of *P. neoaphidis* to the pea aphid *Acyrthosiphon pisum*. Further experiments are required at larger spatial and temporal scales under more natural abiotic conditions to more fully understand the interaction.

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

Liquid media carbon/nitrogen ratio affects the insecticidal activity of the crude soluble protein extract of *Metarhizium anisopliae* 01/58-Su strain against medfly *Ceratitis capitata* (Diptera; Tephritidae) adults

Almudena Ortiz-Urquiza[1]; Ana Borrego[1]; Cándido Santiago-Álvarez[1]; Enrique Quesada-Moraga[1]
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Isolate EAMa 01/58-Su of the Entomopathogenic Fungus *Metarhizium anisopliae* secrets in Adamek's liquid medium a 15 KDa protein showing *per os* toxicity against adults of the medfly *Ceratitis capitata*. The Crude Soluble Protein Extract (CSPE) containing this protein can be used as a natural insecticidal compound against the above pest. However, the commercialization of this natural insecticide goes through the optimization of the production of that toxic protein when culturing the fungus in liquid medium. With this aim we compared the *per os* toxicity of the CSPE from Adamek's liquid medium and other media (A, B, C and D), cheaper than Adamek's, containing different proportion of glucose as a Fermentable Carbon Source (FCS) and yeast extract as a nitrogen source. The Carbon/Nitrogen (C/N) ratios of the assessed media were; A: 0.53/1.00, B 1.33/1.00, Adamek's: 1.99/1.00, C: 2.67/1.00 and D: 5.33/1.00. Fungus growth in terms of Mycelia Dry Weight (MDW) and blastospores production was also monitored through out the fungus culture in all the liquid media. Mycelia and blastospores production were affected by C/N ratio. Our results report that although the higher MDW value was obtained in Adamek's medium, high C/N ratios enhance mycelia production. By contrast low C/N ratios were more suitable for blastospores production. We did not find any *per os* toxicity in the CPSE from media with the highest C/N values, C and D medium, in fact SDS-PAGEof these CSPE did not show the presence of the 15 KDa toxic protein. Toxicity of the CSPE decreased as follow; B medium>Adamek's>C medium, which suggests that although a minimum content of a FCS is required, a high nitrogen content in the medium increases secretion of this insecticidal protein.

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

Viability of formulations of *Beauveria bassiana* for use in grain stores

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Effective biopesticide formulations act to improve the persistence, application and uptake of conidia. Viability needs to be tested during biopesticide development to check that formulating agents do not adversely effect conidial germination. Conidia of two isolates of *Beauveria bassiana*, obtained from beetles in grain stores, were formulated in different carriers, and viability over time was assessed. The formulations included several oils (vegetable and mineral), several powders (including electrostatic powders and bulking agents), one emulsifier and one water based formulation. Formulations were stored at 5°C and 25°C. Results showed the water based formulation had an adverse effect on the viability of both isolates after 1 month at 5°C and after 4 months at 25°C. Conidia formulated in the emulsifier showed excellent viability after 12months for isolate IMI389521, however viability of isolate IMI 386243 dropped off rapidly at 25°C, after less than 1 month and less rapidly at 5°C (over 6 months). Conidia formulated in oil showed excellent germination after 12 months for isolate IMI389521 and after 6months for isolate IMI386243 (experiment still ongoing) when stored at 5°C. Powder formulations showed a similar pattern as oil formulations. Isolate IMI389521 retained better viability over time in the different formulations compared to IMI386243.

CONTRIBUTED PAPERS Monday, 14:00-16:00

MICROSPORIDIA

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

Microsporidian pathogens of the oak processionary moth, *Thaumetopoea processionea* (Lep., Notodontidae), and their potential for inoculative release

Gernot Hoch[1]; Axel Schopf[1]
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Thaumetopoea processionea is an oak pest of high socio-economic impact. Larvae possess urticating setae that cause contact dermatitis and more severe reactions in humans. Infestations in suburban areas and gregarious behavior make inoculative release of microsporidia an interesting option for control of this insect. We studied the occurrence of microsporidia in *T. processionea* larvae from various locations in Eastern Austria using light microscopy. Microsporidia of the genera *Endoreticulatus*, *Nosema*, *Cystosporogenes* and *Vairimorpha* were detected in nine out of 18 populations at prevalence between 1.9% and 15.4%. Spores were isolated and stored in liquid nitrogen. All isolates were tested for infectivity to a laboratory host, *Lymantria dispar*. One *Endoreticulatus* sp. successfully infected *L. dispar* larvae and developed infections like in *T. processionea*. This allowed easy production of inoculum as well as studies with an non-hazardous laboratory host. *Endoreticulatus* infection was restricted to the gut of the host; development of disease was slow but resulted in significantly elevated mortality. Efficient horizontal transmission was shown in the laboratory. We attempted an inoculative release of *Endoreticulatus* sp. by spraying laboratory-produced spores in aqueous suspension on small, isolated oak trees infested with *T. processionea*. Inoculation was successful, however at a low level – maximum prevalence was 9.5%.

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

Effects of a microsporidium from the convergent lady beetle *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae) on three non-target coccinellids

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Convergent lady beetles, *Hippodamia convergens* Guérin-Méneville, are collected annually from their overwintering sites in California for aphid control throughout North America. A microsporidium from *H. convergens* was horizontally transmitted to three non-target coccinellid hosts (*Adalia bipunctata* L., *Coccinella septempunctata* L. and *Harmonia axyridis* Pallas) under laboratory conditions. For all species examined, larval development was significantly longer for microsporidia-infected individuals than for their uninfected cohorts but the microsporidium had no effect on larval mortality. Mean spore counts from smear preparations of infected beetles suggest that the infection was as heavy in *A. bipunctata* (a native coccinellid) as it was in *H. convergens* (the natural host) but lighter in the introduced species *C. septempunctata* and *H. axyridis*. Fecundity and longevity of microsporidia-infected *H. convergens* females were significantly lower when compared to uninfected females. Significant differences in fecundity and longevity were not observed for the three non-target coccinellids; however, environmental stresses may help accentuate differences in fitness between uninfected and microsporidia-infected individuals. When examined during 90-day trials, 100 % vertical transmission of the pathogen was eventually observed for all of the coccinellid species examined.

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

Ultrastructure and pathology of a microsporidium from the convergent lady beetle, *Hippodamia convergens* Guerin-Meneville

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Convergent lady beetles, *Hippodamia convergens* Guerin-Meneville, are collected from their overwintering sites in California and released for aphid control throughout North America. The use of *H. convergens* for biological control has continued for almost a century despite reports that field-collected beetles may be infected with microsporidia. *Nosema hippodamiae* is thought to be the only microsporidium to infect *H. convergens*; however, *N. tracheophila* and *N. coccinellae* are known to infect other lady beetles. These three microsporidia show similar physical characteristics in regards to spore shape and size; however, some variability is seen in regards to the tissues that they infect. Recently, an unidentified microsporidium found in *H. convergens* was transmitted to several coccinellid hosts, suggesting that this pathogen may have a relatively broad host range. Host overlap and similar pathogen characteristics raise questions regarding the true identity of the microsporidia that infect coccinellids. The aim of this study is to provide information on pathogen ultrastructure and tissue pathology of the unidentified microsporidium in *H. convergens*. Uninfected and microsporidia-infected *H. convergens* will be examined by transmission electron microscopy and this information will help provide the basis for a formal description of the pathogen.

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

Life cycle of a microsporidian isolate (*Nosema* sp.) from the three spot grass yellow butterfly, *Eurema blanda arsakia*

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A microsporidium isolated from the larvae of the three spot grass yellow butterfly, *Eurema blanda arsakia*, was named as an EB isolate. This microsporidian isolate possesses the molecular and morphological characteristics of the genus *Nosema*. The *in vitro* propagation system of this isolate in IPLB-LD652Y (*Lymantria dispar*) and NTU-LY (*L. xylina*) cell lines were successfully established. The development of this microsporidium under the *in vitro* cultivation was observed. This isolate was not only developing in the host cytoplasm but also found to develop in the nucleus of the host cell. Interestingly, both meront and sporont could internalize the host cell components during their development, and the EB isolate could be clearly stained by the mitochondrion specific dye. Where did those mitochondrial components come from and what's the function of them are still questions need to be elucidated.

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

A new species, *Vairimorpha ocinarae* n. sp., isolated from *Ocinara lida* Moore (Lepidoptera: Bombycidae) in Taiwan

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A new microsporidium was isolated from Ocinara lida Moore in Taiwan. The isolate causes systemic infection of larvae. The midgut epithelium, Malpighian tubules, and midgut muscle were main target tissues for this species. The different developmental stages of this isolate have been found, and the characteristics of the stages were corresponded to that of the genus Vairimorpha, except for the atrophied fat bodies. This isolate is different from the previous homologous isolate, Endoreticulatus sp. which is found only in the midgut epithelium. Binuclear spores and octospores both possessed exhibited 12 coils of polar tube. The electron-dense granules were the main type of episporontal inclusions and different from other Vairimorpha spp. except for V. sp. NIS-M11 and V. invictae. Based on the phylogenetic analysis of SSUrDNA sequence, this isolate is also closely related to V. sp. NIS-M11 and V. sp. NIK-3h that isolated from Bombycidae. According to the characteristics of morphology and molecular marker, this new isolate should be named Vairimorpha ocinarae n. sp.

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

A new microsporidian species isolated from the freshwater shrimp, *Caridina formosae*

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The new microsporidium was isolated from the endemic, Taiwanese, atyid shrimp, *Caridina formosae* (Decapoda, Atyidae) from northern Taiwan. The conspicuous symptom of the infected shrimp is the opaque, white xenomas, found usually around the alimentary canal in the haemocoel, the dorsal part of abdomen underneath the dorsal median carina, and on the gill. A fully developed xenoma consisted of a hard and thick capsule and filled with sporophorous vesicles. The parasites within a sporophorous vesicle were synchronous in

development, while among sporophorous vesicle were different in development stages. Fresh spores were pyriform, measuring 6.53 x 4.38 μm. The spore contained a nucleus and isofilar polar-filament with 9-11 coils. The phylogenetic analysis of small subunit rDNA showed that this isolate is closely related to the species of the genus *Dictyocoela*, a group of microsporidia from crustacean. However, the identities of the SSUrDNA sequences were only around 81%. Therefore, we propose that this isolate is a new species but needs more morphological and molecular evidences to clarify taxonomic position.

Contributed paper. Monday, 15:30. **22**

Rapid DNA extraction from microsporidian spores of insect origin

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Extraction of DNA from the microsporidian spores of insect origin has required an involved process using agitation with a bead beater or other additional procedures. We located a commercial DNA extraction kit, with which we were able to extract the DNA from the spores of the *Nosema*, *Vairimorpha*, and *Endoreticulatus* species. The optimum heat treatment time is more than twice that of the original manufacturer's suggestion. More than 10^8 spores per reaction produced a higher efficiency of DNA yield, and the minimum quantity of the spores per reaction is approximately 100 spores for amplifying SSUrRNA. The kit requires minimal procedures and saves time for the DNA extraction of microsporidian spores, facilitating DNA analyses.

Contributed paper. Monday, 15:45. **23**

Prevalence rates and genetic diversity of microsporidia associated with European corn borer *Ostrinia spp.* (Lepidoptera: Crambidae) in France

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Samplings of natural populations of European corn borer (ECB) *Ostrinia spp.* collected in France were examined for microsporidian infection. Parallel light microscopic and PCR-based detection carried out for 30 ECB larvae resulted in 30 and 57% parasite's prevalence rate, respectively. PCR-based detection using SSU rRNA primers could yield specific product even at 1000-fold dilution of initial infected host DNA sample. Further PCR-detection of microsporidia was performed using *O. nubilalis* larvae collected from maize (N=602) and *O. scapulalis* larvae collected from mugwort (N=286) in 2000-2003. Microsporidia infection levels of larvae from both maize and mugwort were low, although some were present in both species. Among six samples of 40-50 larvae from mugwort, infection was found only in one population with 8% prevalence rate. PCR products (1123 b.p.) from two samples were sequenced and showed 99.9% similarity to *Nosema pyrausta* SSU rRNA. Among 12 samples of 47-50 ECB larvae from maize, microsporidia were found in 7 populations with prevalence rate ranging from 2 to 10%. Of 10 PCR products sequenced, six showed highest (99.9%) similarity to *Nosema pyrausta*, three – (96%) to *N. bombi*, and one – (82%) to *Encephalitozoon hellem*. Supported by RFBR no. 07-04-92170, no. 07-04-00269; CNRS, PICS no.3864; and RF President's grant no. MK-653.2007.4.

CONTRIBUTED PAPERS Monday, 14:00-15:00

NEMATODES 1

Contributed paper. Monday, 14:00. **24**

Development and ultrastructure of the bacterial receptacle in *Steinernema* nematodes (Nematoda: Steinernematidae)

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Infective juveniles of *Steinernema* spp. are colonized by a monoculture of *Xenorhabdus* bacteria at a discrete structure located in the anterior portion of the intestine known as the 'bacterial receptacle'. Early studies indicate this structure is apparently a modification of the ventricular region of the intestine that lies right beneath the esophago-intestinal valve in the nematode IJs. Until know, no study has been undertaken to confirm the origin and/or histological nature and development of this structure. This is a critical step to help determine the processes that lead to its formation and also to the interactions between *Xenorhabdus* bacteria and their nematode hosts in this particular portion of the nematode's alimentary tract. In this study we considered *S. carpocapsae* (ALL Strain) as a model system to study the development of the intestinal receptacle. Observations were initiated with the examination of embryos (picked up at one-cell stage) mounted on agar slabs (about 0.5 mm thick)followed by first stage juveniles and carried on to adult stages. A motorized inverted Olympus IX81 microscope equipped with a time-lapse imaging systemand interference contrast optics (DIC) was considered for this study. Transmission electron microscopy (TEM) studies were also conducted to investigate the histological nature of this structure.

Contributed paper. Monday, 14:15. **25**

Biochemical and molecular characterization of symbiotic bacteria of four *Steinernema* from Costa Rica, *S. costaricense* n.sp.(CR9), *S. puntauvense* n. sp. (Li6), *S. websterii* (CR5) and *Steinernema sp.* (T4)

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Four *Xenorhabdus* spp. were extracted from newly recovered Costa Rican *Steinernema* species: *S. websteri* (CR5), *S. costaricense* n. sp. (CR9), *S. puntauvense* n. sp. (Li6) and *Steinernema* sp. (T4). These four *Xenorhabdus* isolates were characterized by biochemical traits and sequence analyses of the 16S rDNA gene. Similarity matrices were calculated and cluster analyses were performed by UPGMA method. The derived dendrogram based on phenotypic traits placed the four Costa Rican *Xenorhabdus* isolates into three different clades, with *S. websterii* symbiont in one clade, T4 symbiont in another clade alone, and *S. puntauvense* and *S. costaricense* symbionts placed in a third clade, but belonging to two different clusters. *S. puntauvense* symbiont was more closely related to *X. bovienii*, and the *S. costaricense* symbiont was positioned in a separate cluster. Sequence analyses of 16S rDNA genes confirmed *Xenorhabdus* CR5 is 96% identical to *X. nematophila* ('RIOBRAVIS'), *Xenorhabdus* Li6 had a 95% of similarity with *X.*

bovienii, CR9 shared 95% of similarity with *X. szentirmaii* and T4 95% with a *Xenorhadus* sp. strain. Since results were not conclusive for species identity (less than 97%), further analyses is required to find if the T4, CR9 and Li6 isolates represent new species of *Xenorhabdus* genera.

Contributed paper. Monday, 14:30. **26**

Bacillus bacteria and their fitness consequences on _Pristionchus_ nematodes

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The nematode *Pristionchus pacificus* is a genetic and molecular model system in evolutionary biology and recently its genome has been sequenced at 10X coverage. Currently we are developing *P. pacificus* as a system to study bacterial pathogenicity. *P. pacificus* and other *Pristionchus* species are associated with scarab beetles. For example, *P. entomophagus* is found on dung beetles and *P. pacificus* on oriental beetles. Using large-scale 16S sequencing of bacteria isolated from nematodes from beetles and soil we discovered that these nematodes associate with a range of *Bacillus* species. We decided to investigate avoidance behavior of *P. pacificus* and pathogenicity of *Bacillus*. *Pristionchus* displays unique chemoattraction profiles when exposed to a number of commercial and naturally isolated *Bacillus* species and is highly repulsed by *B. thuringiensis* and another species designated *Bacillus* sp. 1. Both species do not cause mortality but causes slow development and low fecundity. Using the forward and reverse genetic platform available for *P. pacificus* we are investigating the molecular mechanisms involved. We have also started large-scale sampling of *Bacillus* from a range of habitats to test pathogenicity to *P. pacificus* and compare with *Caenorhabditis elegans*, which differs in morphology and gene machinery.

Contributed paper. Monday, 14:45. **27**

Suppressive effects of metabolites from _Photorhabdus_ spp. and _Xenorhabdus_ spp. on phytopathogens of peach and pecan

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Our objective was to determine the suppressive abilities of bacterial metabolites derived from *Photorhabdus* and *Xenorhabdus* spp.on *Glomerella cingulata*, *Phomopsis* sp., *Phytophthora cactorum*, and *Fusicladosporium effusum*, which are fungal or oomycete pathogens of pecan, and *Monilinia fructicola*, a fungal pathogen of peach. Based on *in vitro* assays, we concluded that metabolites derived from two strains of bacteria, *P. luminescens* (VS) and *X. bovienii* (SN) were superior in potency compared with others tested. In *in vivo* tests, 6 or 12% dilutions of *P. luminescens* (Hb) or *X. bovienii* (SN) metabolites caused 90 to 100% suppression of *P. cactorum* lesions on pecan leaves with only slight phytotoxicity. No phytotoxic effects were observed in detached peach leaves at dilutions up to 25%. Metabolite treatments, derived from *P. luminescens* (Hb) and *X. bovienii* (SN) were also tested for suppression of *F. effusum* sporulation in detached pecan shoots. Reductions in sporulation caused by bacterial metabolites were similar to those following treatment with two chemical fungicides, dodine and fenbuconazole; a third chemical, triphenyltin hydroxide had no effect. Further research is warranted to determine if fungal or oopmycete incited diseases in pecan and peach can be controlled with metabolites of *Xenorhabdus* spp. and *Photorhabdus* spp.

SYMPOSIUM (Div. of Microbial Control) Monday, 16:30-18:30

Utilizing Insect Pathogens in Green Pest Management Systems

Symposium. Monday, 16:30. **28**

The long and winding road – discovery to commercial product: Are we there yet?

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In theory, microbial control strategies offer the most sustainable and ecologically-acceptable means of crop protection. Frequently, research promises elegant solutions to some of our most intractable pest and disease problems. Yet this potential is infrequently translated into success at the operational and commercial level. All too often, the process is then repeated. Is it possible to identify potential roadblocks to the development of a reliable, efficacious and commercially-viable product? Do we need to focus our research efforts less on the discovery end, and more on the delivery side of the equation? What do we know about microbial ecology? More to the point, what don't we know that would make a difference? By looking at past successes, understanding why things fail, understanding the needs of the market and the regulatory environment, and examining what tools, techniques and resources are needed to facilitate the efficient use of microorganisms, we should be able to develop a more rational and successful pathway to commercial success.

Symposium. Monday, 16:50. **29**

Exploring tritrophic interactions: Biological control of an obligate pest by its obligate parasite

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Nematicides have been used to control plant parasitic nematodes, but over the last decade legislative measures have restricted their use as they are amongst the most toxic compounds used in agriculture. Therefore alternative approaches are being explored. These range from the development of resistant varieties and genetic engineering to the development of biological control agents. The life-cycle of plant parasitic nematodes includes two levels of trophic interaction, one between the plant and the parasitic nematode, and another between the nematode and any microbial pathogens present in the soil. Therefore the cuticle is an organ that provides a barrier between the nematode and its environment. The cuticles of plant parasitic nematodes have exhibit inter and intra specific variability with respect to the nematode hyperparasite *Pasteuria penetrans*. Endospores of this Gram positive obligate bacterium can adhere to and infect one strain of nematode but not another. This variation appears to be as great in parthenogenetically reproducing plant parasitic nematodes as in amphimictic reproducing groups. The implications of this variation for the population dynamics of the hyperparasite will be discussed.

Symposium. Monday, 17:10. **30**

Proposals for improved registration requirements for microbial biological control agents

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Micro-organisms used in biological control of insects and diseases must be registered before commercial use. Rules for submission of safety data largely follow those applied for synthetic compounds. Proposals for a more balanced regulation of microbial biocontrol agents are based on a review of their potential risks carried out in 2006 by the EU supported Policy Support Action REBECA. The results developed within the Action are summarized and discussed. Proposals on how to regulate invertebrate biocontrol agents, particularly nematodes, will also be presented.

Symposium. Monday, 17:30. **31**

Use of microbial agents in urban pest management systems

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World population reached 6 billion in 2000, and is projected to grow to 8.9 billion by 2050. Despite the fact that the urban population is about half of the total population, the percentage of land occupied by urban areas is only about three percent. Urban agglomeration frequently results in profound environmental impact, including pesticide pollution issues, and municipalities often have widespread contamination of surface waters due to urban pesticide application. Many urban areas draw their drinking water from surface sources, and concerns about the environmental fate and long-term health effects of pesticides have led city and government groups to pursue less chemically intensive management practices. Urban microbial products are used for management of disease vectors, horticultural, turf and structural pests. Their greatest strength is their safety, as they are essentially nontoxic and nonpathogenic to animals and humans. Because most microbial insecticides are effective against a narrow range of pests and because these insecticides are vulnerable to rapid inactivation, users must properly identify target pests and plan the most effective application. The same qualities mean that microbial insecticides can be used without undue risks of human injury or environmental damage. Consequently, microbial insecticides are becoming important tools in urban insect management.

Symposium. Monday, 17:50. **32**

Conservation biological control strategies with entomopathogenic fungi: Potential and perspectives

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Entomopathogenic fungi are part of the functional biodiversity in agricultural ecosystems and have a valuable contribution to make to sustainable pest management strategies through the ecosystem services they provide. Conservation biological control (CBC), involves '*modification of the environment or existing practices to protect and enhance natural enemies --- to reduce the effect of pests*'. CBC does not rely on the addition of natural enemies but rather on identifying strategies to promote those natural enemies already present within crop ecosystems, based on a thorough understanding of their biology, ecology and behaviour. CBC approaches are applicable to entomopathogenic fungi and in this paper I will provide a background on how entomopathogenic fungi are currently exploited in CBC and then discuss the theory, practice and opportunities available for their further development and utilisation in ecologically-based pest management strategies.

Symposium. Monday, 18:10. **33**

Entomopathogenic nematodes market diversity

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Since their commercialisation, nematodes have been used against barely more than 2 insects for the first 10 years: Sciarid flies and vine weevils. Since the mid-1990s, however, the market diversity of entomopathogenic nematodes has increased considerably. Specific events resulting in the release of research funds triggered market development like the invasion of *Scapteriscus vicinus* and *Diaprepes abbreviatus* in Florida and, more recently, the invasion of the western corn root weevil, *Diabrotica virgifera virgifera*, in Europe. The sudden resistance of some *Cydia-pomonella*-populations against the commercial C.-pomonella-Granulose-Virus (CpGV) was spuring research on the use of nematodes against diapausing larvae in Europe. Few markets have been or are currently developed without preceding triggering events. The control of leafminers (*Liriomyza* spp.) and woodlice (*Porcellio scaber and Armadillidium* spp.) will probably remain small niches. Other spontaneously developed markets are likely to become bigger in the future, like the control of the hazelnut borer (*Curculio nucum*), the buprestid *Capnodis tenebrionis* or the palm weevil, *Rhynchophorus ferrugineus*. The development of new markets benefits from the awareness in the control potential of EPNs. It is thus a self-inforcing process. With growing competition between companies, they are likely to expand their investment in the development of new markets.

CONTRIBUTED PAPERS Monday, 16:30-18:30

BACTERIA 1

Contributed paper. Monday, 16:30. **34**

Structural and mutational analysis of the receptor-binding domain of Cry4Aa mosquito-larvicidal protein

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The Cry4Aa toxin from *Bacillus thuringiensis* is toxic to larvae of *Culex*, *Anopheles*, and *Aedes* mosquitoes, which are vectors of important human tropical diseases. In order to understand the mechanism of toxic action and design modified toxins with improved potency that could be used as effective biopesticides, we determined the structure of this toxin in its functional form by X-ray crystallogaraphy. Like other Cry toxins, the activated Cry4Aa toxin consists of three globular domains, a seven-helix bundle responsible for pore formation (domain I) and the two other domains having structural similarities with carbohydrate binding proteins: a ß-prism (domain II) and a plant lectin-like ß-sandwich (domain III). We also studied the effect on toxicity of amino acid substitutions and deletions in three loops located at the surface of the putative receptor-binding domain II of Cry4Aa. Our results indicate that one loop is an important determinant of toxicity. Moreover, a functional importance of an aromatic amino acid cluster at the surface of Cry4Aa domain II was investigated via mutational analysis. A reduction of toxicity was observed suggesting that this region plays a crucial role for the target specificity and mosquito-larvicidal activity.

Contributed paper. Monday, 16:45. **35 STU**

Effect of the *Bacillus thuringiensis* Cry4Ba toxin on the peritrophic membrane in *Aedes aegypti* mosquito larvae

Seangdeun Moonsom[1]; Urai Chaisri[2]; Ping Wang[3]; Chanan Angsuthanasombat[1] [1]Institute of Molecular Biology and Genetics, Mahidol University, Salaya, Puttamonthon Nakhon Pathom 73170, Thailand, [2]Faculty of Tropical Medicine, Mahidol University, Bangkok 10400, Thailand [3]Department of Entomology, New York State Agricultural Experiment Station, Cornell University, Geneva, NY 14456, USA.
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Bacillus thuringiensis (Bt) Cry4Ba is highly toxic to *Aedes* mosquito larvae. In the mosquito larval midgut, the peritrophic membrane (PM) lines the gut epithelium and serves as a protective barrier against pathogens. To understand the interaction of the PM with the Bt toxin, binding of Cry4Ba toxin to the PM and alteration of the PM in Cry4Ba fed *Aedes* larvae were examined. Fluorescence microscopy of *Aedes* larval PMs incubated with Cry4Ba *in vitro* demonstrated that Cry4Ba bound to the PM and Far Western blot analysis indicated that Cry4Ba could bind to three proteins (21-, 24- and 25-kDa in molecular weight) from the PM. *In vivo* observations of the PM by fluorescence microscopy showed that the PM became permeable to FITC-dextran (MW. 2000 kDa) in *Aedes* larvae treated with Cry4Ba. Furthermore, electron microscopical examinations showed structural changes of the PM, presence of bacteria in the ecto-peritrophic space and damaged microvilli of the midgut epithelium cells in larvae treated with the toxin. These findings suggest that Cry4Ba toxin may act on the PM, leading to alteration of permeability of the PM and consequently weakening the protective function of the PM in mosquito larvae.

Contributed paper. Monday, 17:00. **36 STU**

Inter-molecular interaction between *Bacillus thuringiensis* Cry4Ba and Cry4Aa mosquito-larvicidal proteins in lipid membranes results in enhanced toxicity

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Cry4Aa and Cry4Ba toxins are the mosquito-larvicidal proteins produced from *Bacillus thuringiensis* subsp. *israelensis* (Bti). These two proteins are closely related and share similar three-dimensional structures. Previous study demonstrated the *in vivo* synergism between these two toxins suggesting that they could interact together to enhance their toxicity. In this study, the combined effects of either wild-type or different mutant toxins on biological activity and membrane perturbation were investigated. The increased toxicity against *Aedes aegypti* and *Culex quinquefasciatus* larvae was observed when two mutants, the Cry4Aa domain II mutant (ΔKY439) and the Cry4Ba domain I mutant (N183Q or N183K), were mixed. In the absence of lipid membranes or their toxin-binding proteins, both wild-type and mutant Cry4Aa and Cry4Ba proteins showed no observed inter-molecular interaction. However, the enhancement of membrane perturbation was found when these Cry4Aa and Cry4Ba toxins were mixed indicating that these two toxins could interact in lipid membranes and facilitate the ability to perturb membranes.

Contributed paper. Monday, 17:15. **37 STU**

Functional analysis of the truncated BinA component of the binary toxin from *Bacillus sphaericus*

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Bacillus sphaericus produces binary toxin which is highly toxic against *Culex* mosquito larvae. This toxin consists of two proteins, BinA (42 kDa) and BinB (51 kDa). BinB serves as the specificity determinant by binding to the specific receptor on the epithelium gut cell membrane whereas BinA is required for the toxic action. Previous evidences suggest that interaction between BinA and BinB is necessary before internalization of both components into the cytosol. To identify region in BinA that could be responsible for this interaction, four truncated fragments of BinA were constructed. The 11 kDa and 22 kDa fragments represent the N-terminal part whereas the 17 kDa and 28 kDa were from the C-terminal domain. All truncated fragments were highly produced in *E. coli* as inclusion bodies but showed no toxicity against *Culex quinquefasciatus* larvae when fed either alone or as a mixture with BinB. The *in vitro* binding assay showed strong binding of both C-terminal fragments (17 kDa & 28 kDa) to BinB protein. These results demonstrated that both N- and C-terminal domains of BinA are required for full activity and the C-terminal domain plays an important role during BinA-BinB interaction.

Contributed paper. Monday, 17:30. **38 STU**

Amino acid substitutions in selected regions of *Bacillus sphaericus* BinB toxin revealed residues important for toxicity

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The mosquito-larvicidal binary toxin produced by *Bacillus sphaericus* (*Bs*) is composed of BinB and BinA subunits. Both proteins function together to kill mosquito larvae. BinB is proposed to act as a specific receptor binding component, whereas BinA is important for toxicity. To study the function of amino acids in two regions of BinB that are absent in BinA, four block mutations were constructed. Mosquito-larvicidal activity assays against *Culex quinquefasciatus* larvae revealed that $_{111}YLD_{113}$=>$_{111}AAA_{113}$, $_{115}NNH_{117}$=>$_{115}AAA_{117}$, and $_{143}GEQ_{145}$=>$_{143}AAA_{145}$ caused a slight reduction in toxicity compared to that of the wild type, whereas replacement at $_{147}FQFY_{150}$=> $_{147}AAAA_{150}$ resulted in a total loss of toxicity. To identify residues playing critical role in this region, single amino acid substitutions were performed. Mosquito-larvicidal activity assays revealed that two mutant toxins, F147A and Q148A, showed less toxicity than that of the wild type. However, the mutants F149A and Y150A yielded a total loss of toxicity. Intrinsic fluorescent spectroscopy analyses suggested that all mutant proteins should have similar structures to that of the wild type. Dot blot analysis showed that all mutant proteins could interact with BinA. Taken together, it is possible that F149 and Y150 residues may play an important role for receptor binding of BinB. The receptor binding of mutant toxins compared to the wild type toxin is under investigation.

Contributed paper. Monday, 17:45. **39 STU**

Loop2 in Cry4Aa domain II, but not loops 1 and 3, is essential for the mosquitocidal activity against *Culex pipiens*

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Abstract Cry4Aa is a dipteran specific δ-endotoxin produced by *Bacillus thuringiensis* subsp. *israelensis*. To analyze the role of loops 1, 2 and 3 in domain II of Cry4Aa, a series of mutants in which one of the loops was replaced with either of the other two loop sequences were constructed. Bioassay using *Culex pipiens* larvae revealed that the replacement of loop 2 with loop 1 or 3 caused significant decrease of the mosquitocidal activity, whereas the mutants in which loop 1 and/or loop 3 were replaced showed only marginal decrease of the mosquitocidal activity. These suggested that loop 2 was essential for mosquitocidal activity against *C. pipiens* but loop 1 and 3 were not. Processing experiments using trypsin showed degradation products of the Cry4Aa mutants in addition to the active fragments of 45 and 20 kDa. The level of degradation, however, was not correlated with their mosquitocidal activities, suggesting that the Cry4Aa mutants could act before the detoxification by mosquito midgut proteases. Our results suggested that the loop 2 contributed to the stability of Cry4Aa structure and possibly to the receptor binding.

Contributed paper. Monday, 18:00. **40 STU**

Identification of the midgut binding-molecule for Cry4Ba toxin in *Anopheles albimanus* larvae

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Gram-positive bacteria *Bacillus thuringiensis* (*Bt*) synthesizes highly specific larvicidal proteins as parasporal crystalline inclusions during sporulation. *Bt* subs. *israelensis* (Bti) produces four Cry toxins (4Aa, 4Ba, 10Aa and 11Aa), and two Cyt proteins (1Aa and 2Ba), toxic to mosquito-larvae of the genus *Aedes*, *Anopheles*, and *Culex*. These mosquitoes are serious human disease vectors that transmit dengue virus, malaria, and filarial parasites, respectively. *Anopheles albimanus* is the principal vector for the transmission of malaria in Mexico. Although several anopheline species are poorly controlled by Bti, *A. albimanus* represents an exception to this rule. We have previously shown that toxin Cry4Ba is toxic to 4th instar *A. albimanus* larvae and is bound by a GPI-anchored 70 kDa protein present in midgut brush border membrane vesicles. Now we present evidence that identifies this protein as an a-glucosidase by mass spectrometry and affinity chromatographic analysis. We have cloned the gene coding for this particular a-glucosidase by means of 5´ and 3´ RACE experiments. Its expression will be silenced in order to show its in vivo functional role as a receptor for Cry4Ba toxin.

Contributed paper. Monday, 18:15. **41 STU**

Novel insecticidal crystal protein genes of *Bacillus thuringiensis* strains isolated from soil samples in China

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Bacillus thuringiensis strains F14-1 and YBT-978 were isolated from soil samples in China. A novel Insecticidal crystal protein gene *cry51Aa1* was cloned and characterized from strain F14-1 as 930 bp long, encoding polypeptide of 309 amino acid residues with predicted molecular weight of 34 kDa. When transferred to an acrystalliferous *B. thuringiensis* strain, unlike other relatively small crystal proteins, predominant 34-kDa proteins were expressed and bipyramidal crystals were formed which are the same with that of the wild strain without any other helper proteins, and toxicity to Lepidoptera was detected. From stain YBT-978, *cry7Ba1* was cloned as 3465 bp long, encoding polypeptide of 1154 amino acid residues with predicted mw. of 130.6 kDa. Bipyramidal crystals were synthesized by Cry7Ba1 which could be solubilized only at high pH values (>11.5). Bioassay showed spore-crystal mixture of Cry7Ba1 is not toxic to any Lepidoptera, Dipteral and Coleopteran species that have been tested, while the solubilized crystals in vitro showed obvious toxicity to *Plutella xylostella*. Several recombinants substituting the N-terminal half or C-terminal half of Cry7Ba1 with that of Cry1C and Cry1Ac were constructed, and the solubility test indicated the characteristic solubility of Cry7ba1 depend on its C-terminal half.

CONTRIBUTED PAPERS Monday, 16:30-18:30

VIRUSES 1

Contributed paper. Monday, 16:30. **42**

Phylogenetic approaches to delimit baculovirus species based on single gene and whole genome data

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Baculoviruses are well known insect pathogens and yet we know little of their diversity. The nomenclature of baculoviruses, juxtaposing host name and virus morphology, has long had the advantage of being simple, but it introduces a lot of confusion for taxonomic purposes. The wealth of available baculovirus sequences provides an excellent framework to test a new phylogenetic method that delimits clusters of individual sequences into independently evolving groups or species. We have assembled 3 datasets: 2 based on single genes, 293 polyhedrin and 221 lef-8 sequences, and one based on 43 complete genomes. We use molecular phylogenies to reveal the interrelationships of individual viral isolates. We have developed a new method that detects the transition from between-species to within-population branching in phylogenies. One of the benefits of this approach is the definition of groups of individuals with shared evolutionary histories. These clusters of isolates can be interpreted as species groups. This method provides an objective way to delimit species without a priori assumptions of host use. By comparing the 3 types of datasets, we aim to validate the method for baculoviruses and determine the most appropriate data to use to describe new baculovirus isolates.

Contributed paper. Monday, 16:45. **43**

Genome sequence of the complete genotype of *Spodoptera frugiperda* multiple nucleopolyhedrovirus isolate from Nicaragua

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To understand the molecular basis for differences in speed-of-kill phenotypes the genome sequence of a fast-killing *egt* minus genotype of a *Spodoptera frugiperda* multiple nucleopolyhedrovirus from the USA (SfMNPV-3AP2) was compared with that of a slower-killing *egt* minus SfMNPV genotype originally isolated in Nicaragua (SfMNPV-NIC). Nucleotide sequences were strongly conserved (99.5% identity) and a high degree of predicted amino acid sequence was observed between the two isolates. The SfNIC-B genome was 132,947 bp, 1,617 bp larger than that of SfMNPV-3AP2, due mainly to a deletion of 1,428 bp located between Sf26 (*egt*) and Sf27 in the latter. A total of 145 open reading frames (ORFs) were identified in SfNIC-B, three of which were absent in SfMNPV-3AP2. In turn, SfNIC-B lacked the SfMNPV-3AP2 ORF129 homologue. Other genes, such as *odv-e66a*, *p26b*, were also truncated in SfMNPV-3AP2 due to small deletions, but lack of these genes has no substantial effects on the biological activity of these viruses. A deletion in the homologous region 8 of SfNIC-B was also observed. Construction of recombinant viruses that will help determine the genes involved in virulence is currently being undertaken.

Contributed paper. Monday, 17:00. **44**

Genomic and host range study of the smallest lepidopteran NPV, *Maruca vitrata* multiple nucleopolyhedrovirus

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The complete genome of the Maruca vitrata multiple nucleopolyhedrovirus (MaviMNPV) isolated from the legume pod borer, Maruca vitrata (Lepidoptera: Pyralidae) was sequenced. It was 111,953 bp long, with an overall 39% G+C content, and contained 126 open reading frames (ORFs) encoding predicted proteins of over 50 amino acids. The gene content and gene order of MaviNPV are most similar to those of Autographa californica MNPV (AcMNPV) and their shared homologous genes are 100% collinear. Except for one ORF (Mv74), all of the MaviNPV ORFs have homologues in the AcMNPV genome. MaviMNPV is the first lepidopteran-specific baculovirus found to be without the homologues of vfgf and odv-e66. In addition, MaviNPV lacks bro (baculovirus repeat ORF) genes that are similar to the AcMNPV ORF 2. Five homologous regions (hrs) were located within the MaviNPV genome, and these contained a total of 44 imperfect palindromes. Phylogenetic analysis of the whole genome revealed that MaviMNPV was separated from the common ancestor of AcMNPV and BmNPV before these two viral species diverged from each other. Moreover, in vitro virus susceptibility experiments revealed that MaviMNPV is partially permissive to IPLB-LD-652Y cells, supporting the representation of MaviMNPV as a distinct species of the group I lepidopteran NPVs.

Contributed paper. Monday, 17:15. **45 STU**

Comparative genomics of different isolates of *Cydia pomonella* granulovirus (CpGV)

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The *Cydia pomonella* Granulovirus (CpGV) isolate CpGV-M1 was one of the first fully sequenced granulovirus genomes. Further CpGV isolates from different geographic origins containing different genotypes had been previously identified by restriction analysis. In the framework of testing different CpGV isolates for improved virulence against codling moth populations with CpGV resistance, the genome of the resistance overcoming isolate CpGV-I12 was sequenced and compared to CpGV-M1 as well as the original CpGV-M isolate, which was completely re-sequenced using pyrosequencing technology. Sequence comparisons between CpGV-M1, CpGV-M and CpGV-I12 revealed only small differences between the three viruses. One difference was found in an insertion of 0.8 kbp in CpGV-I12. The same insertion is also present in the genome of CpGV-E2, an *in vivo*-cloned genotype derived from an English CpGV-E isolate. Interestingly, these insertions show an inverted-repeat structure and are located at different insertion sites in the genomes of CpGV-I12 and -E2. The genome comparisons provide first clues about the virulence factors of CpGV involved in the overcoming of CpGV resistance.

Contributed paper. Monday, 17:30. **46 STU**

A new nucleopolyhedrovirus of *Lymantria xylina* Swinhoe (Lepidoptera: Lymantriidae) with a defective fp25 gene from Taiwan

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A new multiple-nucleocapsid nucleopolyhedrovirus (MNPV) isolated from casuarina moth, Lymantria xylina Swinhoe, (Lepidoptera: Lymantriidae) in Taiwan. This NPV was propagated in IPLB-LD-652Y and NTU-LY cell lines and showed only FP (few polyhedra) cytopathic effect (CPE) in the infected cells. It authenticity was confirmed by sequence analysis and BamHI digestion profiles of the polyhedrin of this virus with those of LyxyMNPV and LdMNPV. Polyhedrin amino acid sequence analysis revealed that this virus belongs to Group II of baculoviruses and is closely related to LdMNPV rather than its homologous LyxyMNPV. Several other important genes of this virus had been cloned and sequenced for phylogenetic analysis. Similarly, this virus is closely related to LdMNPV as the analysis of polyhedrin. A significant deletion of fp25k sequence of this virus was found, 44 bps deletion was compared to that of LdMNPV or LyxyMNPV, this deletion may play an important role on FP cytopathic effect. In ultrastructure observation, the nuclei of the infected LD cells contain few polyhedra, one or two OBs (occlusion body) in a nucleus, and filled with free nuclocapsids and viruses. This virus genome size was approximately 139 kbs which is smaller than that of LdMNPV and LyxyMNPV, and the RFLP (restriction fragment length polymorphism) profiles of whole genome indicated that this virus was quite different from that of LdMNPV or LyxyMNPV. These results showed that this isolate is a distinct isolate but closely related to LdMNPV and named LyxyMNPV-2.

Contributed paper. Monday, 17:45. **47**

Sequence and organization of the *Orgyia leucostigma* nucleopolyhedrovirus genome

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Orgyia leucostigma nucleopolyhedrovirus (OrleSNPV) is a naturally-occurring viral pathogen of the whitemarked tussock moth (*Orgyia leucostigma*, Lymantridae: Lepidoptera) and has been shown to cause the collapse of past *O. leucostigma* outbreak populations in Nova Scotia, Canada. OrleSNPV was originally field collected from *O. leucostigma* larvae from that province. OrleSNPV DNA was purified and the entire genome sequenced. The OrleSNPV genome is 156,179 base pairs (bp) with a G+C content of 39.9 %, encoding 135 putative ORFs, one of which is unique to OrleSNPV. The three OrleSNPV hrs are interspersed in the latter half of the genome and have a common repetitive element with a consensus sequence of 32 bp. OrleSNPV contains six direct AT-rich repeat regions with two to 10 copies of direct tandem repeat sequences ranging in size from 31 to 97 bp. The presence of an F-protein homologue and results from genome arrangement, amino acid identity, gene parity plot and phylogenetic analyses, place OrleSNPV in NPV group II. The baculoviruses most closely related to OrleSNPV are the NPVs of *Ecotropis obliqua* (EcobNPV), *Chrysodeixis chalcites* (ChchNPV) and *Lymantria dispar* (LdMNPV).

Contributed paper. Monday, 18:00. **48**

Comparative analysis of the genome sequence of two isolates of *Glossina pallidipes* salivary gland hypertrophy virus (GpSGHV) from Uganda and Ethiopia

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The *Glossina pallidipes* salivary gland hypertrophy virus (GpSGHV) causes salivary gland hypertrophy (SGH) and significantly reduces the fecundity of the infected flies. Despite the high prevalence of asymptomatic virus infection in the tsetse colony originating from Uganda at the Seibersdorf Laboratory, the prevalence of symptomatic infection is around 10% and the colony has been stable for the last 20 years. Another tsetse colony originating from Ethiopia was successfully established in the laboratory but subsequently declined and disappeared by 2002 with up to 85% of adults displaying SGH. To better understand the molecular basis underlying the pathogenesis of the Ethiopian virus strain (EVS) its genome was isolated, sequenced and its sequence compared to that of the Uganda virus strain (UVS) previously published (Abd-Alla et al, J. Virol 2008). Both genomes are double-stranded circular DNA molecules with a A+T content of 72% and share 93% similarity at the nucleotide level. The USV genome is 190,032 bp and contains 160 non-overlapping ORFs distributed equally on both strands. The ESV genome is 189,769 bp and has 152 ORFs with 4 ORFs deleted and two cases of ORF fusion. 80% of its ORFs have homologs in the USV genome, 77% have the same length and 72% have synonymous and non-synonymous mutations.

Contributed paper. Monday, 18:15. **49**

Comparative analysis of viruses that cause salivary gland hypertrophy in *Glossina pallidipes* (GpSGHV) and *Musca domestica* (MdSGHV)

Alejandra Garcia-Maruniak[1]; Adly M. M. Abd-Alla[2]; Andrew G. Parker[2]; Tamer Z. Salem[1]; Verena-Ulrike Lietze[1]; Monique M. van Oers[3]; James E. Maruniak[1]; François Cousserans[4]; Alan S. Robinson[2]; Just M. Vlak[3]; Max Bergoin[4]; Drion G. Boucias[1]
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Recently, the genome sequences of the *Glossina pallidipes* and *Musca domestica* salivary gland hypertrophy viruses (GpSGHV and MdSGHV) have been published. Both viruses share general characteristics with the non-occluded insect nudiviruses, such as being insect-pathogenic, having an enveloped, rod-shaped morphology, and possessing a circular dsDNA genome. Although both viruses induce similar disease symptoms, they have distinct structural and molecular characteristics. MdSGHV, measuring 75 by 650 nm, contains a 124,279 bp genome (~44% G+C content) that codes for 108 open reading frames (ORFs). GpSGHV, measuring up to 1.3 µm in length, contains a 190,032 bp genome (28% G+C content) coding for 160 ORFs. The comparative analysis of their genomes showed that 45 MdSGHV ORFs have homology to GpSGHV ORFs while 52 GpSGHV ORFs were homologous to MdSGHV ORFs. However there were genome segments where no homology was found. The phylogenetic analysis of specific genes resulted in the clustering of the two SGHVs separate from the nudiviruses and baculoviruses. In addition to genetic differences, there are numerous pathological differences between the GpSGHV and MdSGHV that may reflect adaptations to their respective dipteran hosts systems. The detailed comparison of their genomes may provide a platform to decipher the basis of these pathological differences.

NEMATODE DIVISION WORKSHOP Monday, 20:00-21:30

Nematode-Bacterium Associations

Workshop paper. Monday, 20:00. **50**

Nematode-Bacteria Symbiosis Research Network: Intertwining knowledge and research tools

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Intimate associations between microbes and eukaryotes are widespread in nature, occurring in every type of ecological niche. The spectrum of such interactions ranges from highly integrated obligatory symbioses to 'loose' associations. One of the most common eukaryote-prokaryote interactions is that between nematodes and bacteria. The range of associations between nematodes and bacteria is incredibly broad, ranging from fortuitous to obligate and from beneficial to pathogenic. At present numerous researchers worldwide are studying associations between these two groups of organisms, but these scientists occupy many different disciplines, and often do not interact. The scope of such research is mostly dictated by nematode trophic groups. Not surprisingly, these researchers come from diverse backgrounds in medicine and veterinary science, entomology, plant biology, genetics etc., yet to date no common coherent ground exists connecting the science being done in this discipline, despite the fact that advances in each

will undoubtedly inform the others. In this presentation I will discuss an ongoing project on a research coordination network on 'Nematode-Bacteria Symbioses" which main goal is to foster interdisciplinary collaborations between scientists and to encourage scientists engaged in basic and applied research to explore how cross-talk and networking can enhance and advance science in this field.

Workshop paper. Monday, 20:15. **51**

Evolution and genetics of *C. elegans*-pathogen interactions

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Over the last decade, the nematode *Caenorhabditis elegans* has become an important model for the study of host-pathogen interactions. Two types of defences appear to be of particular importance: the innate immune system and behavioural avoidance of pathogens. Both defences are expressed against diverse pathogenic microorganisms and they are based on surprisingly complex molecular mechanisms. During my presentation, I will summarize our current understanding of the evolution and genetics of *C. elegans* defences with a particular focus on those directed against *Bacillus thuringiensis*.

Workshop paper. Monday, 20:30. **52**

Innate immunity in nematodes and somaclonal cuticle variation as revealed by *Pasteuria penetrans*

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The IGF1 signalling pathway in mammals is related to the DAF2 pathway in *Caenorhabditis elegans* and influences, amongst other things, fecundity and longevity. It is also involved in the worm's innate immune system. Changes to the cuticle surface of *C. elegans srf* mutants have been shown to affect microbial adhesion and the worm's innate immune system has been implicated. Peptides inhibitory to IGF1 have been shown to alter *C. elegans* fecundity and longevity and were tested to see if they had any effect on the attachment of *Pasteuria* endospores to second-stage juvenile cuticle of root-knot nematodes. Preliminary experiments suggest that the attachment of endospores to juveniles exposed to peptides significantly affected attachment at 18 – 21 hours post-exposure. This result shows the potential importance of innate immunity in generating functional variation to the cuticle surface that affects *Pasteuria* adhesion. The result will be discussed as a possible mechanism for generating somaclonal cuticle variation.

Workshop paper. Monday, 20:45. **53**

The obligate *Wolbachia* endosymbiont in filarial nematodes provides potential targets for disease intervention

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Filarial parasites (*Brugia malayi*, *Onchocerca volvulus*, *Wuchereria bancrofti*) are causative agents of elephantiasis and African river blindness. Current anti-filarial chemotherapy can interrupt transmission by killing the larvae but is less effective on adult worms, which live 10-15 years in humans. There is an urgent need to develop adulticides. The obligate endosymbiont *Wolbachia* is recognized as a potential target for filarial nematode life cycle intervention, as evidenced by loss of worm fertility and viability upon antibiotic treatment, both *in vitro* and *in vivo*, including human trials. However, current antibiotic treatments are not practical due to the dosages and length of treatments that are required. We have been using the genome sequence of *Wolbachia* and comparative genomics to identify potential drug targets. For example, heme biosynthesis was identified as a potential target set due to its presence in the *Wolbachia* genome sequence but its surprising absence from the host *B. malayi* genome and its potential role in worm molting and reproduction. We have therefore undertaken the cloning, overexpression and analysis of the enzymes of this pathway in preparation for drug targeting. We will also provide a progress report on other targets and on informatic approaches to drug target screening.

Workshop paper. Monday, 21:00. **54**

***Photorhabdus:* Molecular analyses of pathogenicity and mutualism**

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Photorhabdus is a genus of entomopathogenic Gram-negative, motile bacteria which belongs to the family Enterobacteriaceae. *Photorhabdus* form a specific mutualistic association with entomopathogenic nematodes of the family Heterorhabditis. The *Photorhabdus* life-cycle is complex but the successful continuation of the mutualism with the nematode depends on the colonisation of the infective juvenile (IJ), a specialist free living stage in the nematode life-cycle. There is evidence to suggest that *Photorhabdus* may form a biofilm in the guts of these IJs and the objective of this study is to investigate whether a link exists between biofilm formation and colonisation. Screening a *P. luminescens* TT01 transposon mutant library resulted in the identification of 8 mutants that showed significantly reduced levels of IJ colonisation. Notably 5 of these mutants were also affected in biofilm formation. The majority of these mutants were identified as insertions in genes predicted to be involved in lipopolysaccharide biosynthesis, supporting our previous findings that O-antigen is required for IJ colonisation. However, one of the mutants involved a transposon insertion affecting *hdfR*, which encodes a LysR type transcriptional regulator. In this study we elucidate the role of *hdfR* in both biofilm formation and IJ colonisation in *Photorhabdus*.

VIRUS DIVISION WORKSHOP Monday, 20:00-21:30

Invertebrate Virus Discovery

Workshop paper. Monday, 20:00. **55**

Hunting for insect pathogens: A genomics approach

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Emerging methods within the field of genomics have increased the number of insect pathogens being discovered and characterized each year. These pathogens provide a rich resource for biological control agents, gene expression systems, and other molecular tools. Using Metagenomics, and gene expression analyses provided the means to identify several viral pathogens such as three ssRNA viruses from the glassy-winged sharpshooter, *Homalodisca vitripennis*; an iridovirus from the whitefly, *Bemisia tabaci*; a dsRNA virus from the Asian citrus psyllid, *Diaphorina citri*; and two ssRNA viruses from fire ants, *Solenopsis invicta*. Many of these emerging insect viruses are single-stranded RNA viruses within the insect Picorna-like viruses. Discovery of new viruses advance taxonomic classifications by providing enough members to create new families, such as Dicistroviridae. Application of the methods used in the discovery of insect viruses, such as cell cultures, transmission electron microscopy, cDNA libraries and gene sequencing are discussed.

Workshop paper. Monday, 20:30. **56**

Discovering nucleopolyhedrovirus and iridescent viruses of *Spodoptera* spp.

Trevor Williams[1]; Oihane Simón[2]; Gabriel Clavijo[2]; Delia Muñoz[2]; Rosa Murillo[2]; Primitivo Caballero[2]; Robert D. Possee[3]; Noe Hernández[4]; Jorge E. Ibarra[4]; Miguel López-Ferber[5]
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Finding viruses in insects is usually easy. Knowing what to do once you have found them is more of a challenge. Work in our laboratories in Spain, France and Mexico has identified considerable inter-isolate variation and intra-isolate diversity in the genotypes of a nucleopolyhedrovirus (SfMNPV) that infects the noctuid, *Spodoptera frugiperda*. An isolate of SfMNPV from Nicaragua comprises at least nine genotypes and their interactions affect key aspects of virus transmissibility, including infectivity, speed of kill and OB production. Eight of these genotypes have deletions of different lengths and we are now beginning to understand the genetic basis for the observed phenotypes which depend of the presence or absence of certain genes, notably *pif* and *p29*. We will compare the diversity observed in SfMNPV with that of the closely-related SeMNPV from *S. exigua* and present the hypothesis that sustained diversity improves the survival of populations of these viruses. Finally, we present a glimpse of the diversity present in populations of an iridescent virus from *S. frugiperda* that is remarkably similar to the genetic variation of iridescent virus infections seen in other species of insects.

FUNGUS DIVISION WORKSHOP Monday, 20:30-21:30

Molecular Phylogenetic Identification Resources for *Beauveria* and *Metarhizium*

Workshop paper. Monday, 20:30. **57**

Web-based molecular phylogenetic identification resources for *Beauveria* and *Metarhizium*

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A continuing impediment to the study of Beauveria, Metarhizium and other entomopathogenic fungi is the lack of clearly defined species boundaries and taxonomies that recognize the cryptic speciation that has occurred in these taxa. We introduce a publicly available web site, to be hosted at the USDA-ARS Systematic Mycology and Microbiology Laboratory, (Beltsville, MD), that provides standardized species identification resources for Beauveria and Metarhizium. The core of this resource is a searchable BLAST database of sequence records for selected isolates representative of the species diversity of these genera based on recently completed and ongoing molecular systematic revisions. All isolates in the database are vouchered in publicly accessible culture collections. Users input query sequences for one of several phylogenetically informative loci that have been determined for all currently recognized species in Metarhizium and Beauveria (e.g., Metarhizium: EF-1alpha, RPB1&2, B-tubulin; Beauveria: ITS, EF-1alpha, Bloc). Descriptions of conserved PCR and sequencing primers to all diagnostic loci will be available. Complete morphological and molecular species descriptions with images will be provided, as well as keys to species. In the future, the website will be expanded to include additional genera of entomopathogenic fungi, and updated as data for new and revised species are published.

MIOCROSPORIDIA DIVISION WORKSHOP Monday, 20:30-21:30

Use of QPCR to Quantify Microsporidia Infection

Workshop paper. Monday, 20:30. **58**

Quantifying developing *Thelohania solenopsae* infections in the red imported fire ant, *Solenopsis invicta*

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The process by which *Solenopsis invicta* becomes infected with the microsporidian *Thelohania solenopsae* in nature is currently unknown. Quantitative PCR may provide a tool capable of studying the epidemiology of the *Thelohania solenopsae* infection. Development of such a method is discussed.

Workshop paper. Monday, 21:00. **59**

Prevalence and levels of *Nosema ceranae* in healthy and declining honey bee colonies

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Nosema ceranae is a worldwide parasite of honey bees that has shown dramatic range expansion in recent years. *N. ceranae* levels can be quantified using qPCR with both ribosomal and protein-coding genes, and we are using these techniques along with histology to clarify the interactions between this pathogen and bee hosts. An analysis of honey bee samples collected between 1995 and 2007 from 12 U.S. states showed that *N. ceranae* has surpassed congener *N. apis* as the predominant microsporidian infection of *A. mellifera* in the U. S. Tissue tropism of *N. ceranae* in the host was quite different from that of *N. apis.* Specifically, while *N. apis* is largely confined to the gut epithelium, *N. ceranae* was found not only in the primary infection site, the midgut, but also in the hypopharyngeal glands, salivary glands, Malpighian tubules and fat body. The complex biological features and disease importance of *N. ceranae* in honey bees invite further research. New tools for measuring gene expression and an effort to sequence and annotate the *N. ceranae* genome should help clarify the means by which this microsporidian affects honey bee health, and the counter-defenses used by bees.

TUESDAY - 5 August

SYMPOSIUM (Div. of Fungi) Tuesday, 8:00-10:00

Virulence Factors in Fungal Pathogens: A Comparative Approach

Symposium. Tuesday, 8:00. **60**

Pathogenicity determinants of the entomopathogenic fungi *Metarhizium anisopliae*

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Our long term goal is to determine the role of all genes involved in an insect pathogens response to an insect host. We have used an array of ESTs representative of a significant fraction of the entire genome to assay expression changes during infection and provide insight into the very intricate mechanisms by which *M. anisopliae* has adapted to survive in the cuticle and hemolymph. Construction of deletion strains for highly expressed genes has led to the identification of a cell surface protein that functions in immune evasion, separate adhesins essential for binding to insect cuticle and plant surfaces, a perilipin that regulates lipolysis, osmotic pressure and formation of infection structures and a protein kinase A that regulates expression of some secreted virulence factors. We have also employed an antisense silencing technique to modulate an osmosensor that signals to penetrant hyphae that they have reached the haemocoel. Exploiting microarrays and gene disruption has therefore been successful in identifying key aspects of pathogenicity.

Symposium. Tuesday, 8:24. **61**

Attenuation of virulence in entomogenous fungi

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Entomogenous fungi like *Metarhizium anisopliae* and *Beauveria bassiana* become attenuated when successively subcultured on artificial media but virulence is restored when they are passaged through an insect host. Exactly why fungi lose virulence when maintained on artificial media is unclear. Nutrition influences the carbon and nitrogen composition of conidia, germination rate and levels of spore bound Pr1; these parameters can to some extent predict the virulence of the inoculum. The importance of these strain-independent parameters as regards the production and quality control of inoculum in commercial systems is discussed.

Symposium. Tuesday, 8:48. **62**

Developing insect models to study the evolution of fungal pathogens

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The study of human diseases requires the testing of microorganisms in model systems. Although mammals are typically used, we argue the validity of using insects as models in order to examine human diseases, particularly the growing number of opportunistic microbes. Insect models may also be used to examine the evolutionary processes involved in the acquisition of virulence factors and host-jumping mechanisms indispensable to emerging pathogens. The evolution of host specialization in pathogens is a topic of considerable interest, particularly since it can represent a decisive step in the emergence of infectious diseases. We used the opportunistic fungus Aspergillus flavus that is capable of infecting a wide variety of hosts, including plants, insects and mammals, although with low virulence. We describe the derivation of an A. flavus strain that exhibited host restriction to insects. The host restriction was shown to be due to nutritional dependence on the insect. An association between this strain and a decreased host range emphasizes the role of nutrition in the host–pathogen relationship with respect to host restriction and evolution towards obligate pathogenesis.

Symposium. Tuesday, 9:12. **63**

Investigating the biology of plant infection by the rice blast fungus *Magnaporthe grisea*

Martin J. Egan[1]; Michael J. Kershaw[1]; Diane O. Saunders[1]; Elise Lambeth[1]; Ana-lilia Martinez-Rocha[2]; Nicholas J. Talbot[1]
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During plant infection, the rice blast fungus elaborates a specialised infection structure known as an appressorium. This unicellular, dome-shaped structure generates turgor that is translated into mechanical force to allow rupture of the rice cuticle and entry into plant tissue. We set out to explore whether the development of a functional appressorium was linked to the control of cell division. This was based on the observation that following germination of a conidium on the rice leaf surface, a single round of mitosis always occurs during germ tube elongation, prior to the formation of an appressorium. We found that blocking completion of mitosis prevented appressorium morphogenesis. Furthermore, we found that following mitosis, conidia always undergo cell collapse and cell death, which appears to be a programmed, autophagic process. Deletion of *MgATG8* prevented autophagy in *M. grisea* and rendered the fungus non-pathogenic. Taken together, our results indicate that appressorium morphogenesis requires genetic control by completion of mitosis and autophagic cell death of the conidium. We have also recently demonstrated the appressorium morphogenesis is accompanied by a burst of reactive oxygen species. Deletion of *NOX1* or *NOX2* which encode NADPH oxidases is sufficient to prevent plant infection by interfering with appressorium function.

Symposium. Tuesday, 9:36. **64**

Are there overlaps between virulence factors of fungal pathogens of arthropods, plants, and vertebrates?

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Fungi inhabit a diverse array of environments as saprophytes, endophytes, and pathogens, each niche presumably requiring unique strategies for adaptation and competition. Survival strategies vary depending on the environment and the degree of competition with other organisms. However, fungi in diverse environments share many of the same developmental characteristics and, in some cases it has been shown, a highly similar complement of genes, whether they are growing in soil, in plants, or in animals. Fungal pathogens of plants, arthropods, and vertebrates may use similar developmental mechanisms and degradative processes to derive and utilize nutrients from distinct sources. Parallels between the methods used to colonize and alter host physiology are evident in both plant and

animal fungal pathogens. Additionally, common signalling cascades regulating virulence have been revealed. However, there appear to be few universal fungal virulence factors described to date, perhaps in part because of the lack of in-depth studies of comparable developmental stages across a range of fungus-host pathosystems. An overview of the current literature, with a focus on specific pathosystems, will be presented.

CONTRIBUTED PAPERS Tuesday, 8:00-9:45

MICROBIAL CONTROL 1

Contributed paper. Tuesday, 8:00. **65**

Bioinsecticide based on *Bacillus thuringiensis* subsp. *kurstaki* delta-endotoxins for the control of the lepidopteran olive tree pathogenic insect *Prays oleae*: From gene cloning to application in the field

Samir Jaoua[1]; Souad Rouis[1]; Slim Tounsi[1]; Nabil Zouari[1]; Imène Saadaoui[1]; Hichem Azzouz[1]; Lobna Abdelkafi Mesrati[1]
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The olive moth *Prays oleae*, is one of the most important insect pests of olives in the Mediterranean basin and spread from Mexico to southern America. The *Bacillus thuringiensis* delta-endotoxins are the most valuable bioinsecticides used currently in commercial agriculture, forest management, and mosquito control**.** They exhibit a high specificity of insecticidal toxicity towards lepidopteran, coleopteran and dipteran insect species. In the laboratory of Biopesticides, 500 strains of *B. thuringiensis* were isolated and many of their *cry* corresponding genes were cloned and characterized, such as cry1Aa, cry1Ac, cry2, cry1Ia, cry4. Although the control of *P. oleae* by *B. thuringiensis* bioinsecticides was attractive, little is known about the mode of action of the correspondent Cry toxins in this insect gut and then selection of adequate bioinsecticides for efficient use and field application. We investigated the role of *B. thuringiensis* endotoxins proteolysis in activation, stability and potency of these toxins towards *P. oleae* compared to other insects and the interaction and organization of the epithelial cells in the larval midgut and the histopathological effects of Cry toxins *in P. oleae* larvae midgut. On the other hand, high frequencies of delta-endotoxins over producing mutants of *B. thuringiensis* were obtained through classical mutagenesis or by genetic engineering of the strains. Besides the genetic and molecular investigations, we developed fermentation processes for economical production of *B. thuringiensis* bioinsecticides based on cheap by-products of local agro-industries. We optimized several media based on gruel- a cheap by-product of semolina factories- and fish meal. High production of bioinsecticides was obtained with a clear overcome of the catabolite repression. On the other hand, we studied possibilities to improve delta-endotoxins production as a consequence of responses of *B. thuringiensis* strains to heat and salt stress leading to toxins production improvement of 66%**.** These alternatives allowed us to scale-up the production of bioinsecticides in 430 litres fermentors. The formulated bioinsecticides were used efficiently for the treatment of 39 olive trees.

Contributed paper. Tuesday, 8:15. **65a**

The influence of *Bacillus thuringiensis* on baculovirus transmission dynamics in the cabbage moth, *Mamestra brassicae*

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Baculoviruses can be combined with other entomopathogens to achieve improved biological control of insect pests. Understanding the population ecology of interacting pathogens and specifically the transmission dynamics in pathogen combinations will assist in predicting the outcome of integrated biological control strategies. We tested the hypothesis that the presence of *Bacillus thuringiensis* subsp. *kurstaki* (*Btk*)in manipulated cabbage moth (*Mamestra brassicae*) field populations would affect the transmission dynamics of *Panolis flammea* nucleopolyhedrovirus (*Pafl*NPV). The combination of spraying *Btk* with *Pafl*NPV resulted in first generation larvae being more likely to be infected with virus when *Btk* was present. The acquisition of baculovirus infection as exposure time increased was found to be highly non-linear. In the presence of *Btk* the number of insects that were able to escape NPV infection was reduced through a combination of changes in host feeding behaviour and delay in onset of host developmental resistance. Viral cadavers in the presence of *Btk* produced significantly lower viral yields compared to those in the absence of *Btk*. When second generation larvae were exposed to these viral cadavers *in situ*, there were significant reductions in subsequent viral mortality. The impact of these results for season long Lepidoptera control are discussed.

Contributed paper. Tuesday, 8:30. **66 STU**

Effectiveness of *Bt* chickpeas and the entomopathogenic fungus *Metarhizium anisopliae* to control *Helicoverpa armigera* (Lepidoptera: Noctuidae)

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The use of transgenic crops expressing lepidopteran-specific Cry proteins derived from the soil bacterium *Bacillus thuringiensis* (*Bt*) is a useful method to control the polyphagous pest *Helicoverpa armigera*. However, as *H. armigera* potentially develops resistance to Cry proteins, the combination of *Bt* and natural enemies such as the entomopathogenic fungus *Metarhizium anisopliae* might be an effective control method. Studies were conducted using a Cry2Aa-expressing chickpea line and a susceptible and Cry2A-resistant *H. armigera* strain. In a concentration-response assay, Cry2A-resistant larvae were more tolerant to *M. anisopliae* than susceptible larvae. In a second bioassay, however, similar mortality levels among the two strains were observed when fed on *M. anisopliae* treated control chickpea leaves. Thus, resistance to Cry2A did not cause any fitness costs that would become visible in an increased susceptibility to the fungus. On *Bt* chickpea leaves, in contrast, susceptible *H. armigera* larvae were more sensitive to *M. anisopliae* than on control leaves. It appeared that sublethal *Bt* damage enhanced the effectiveness of *M. anisopliae*. For Cry2A-resistant larvae the mortality caused by the fungus was similar independent from the food source. It is concluded that *Bt* chickpea plants and *M. anisopliae* are compatible for the control of *H. armigera* larvae.

Contributed paper. Tuesday, 8:45. **67 STU**

The role of population structure in determining *Bacillus thuringiensis* resistance in cabbage loopers, *Trichoplusia ni*

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Low environmental risk and high efficacy of *Bacillus thuringiensis* (*Bt*) for the control of caterpillar pests has led to a dramatic rise in the use of *Bt*. The continued use of *Bt* products in vegetable greenhouses in British Columbia Canada has, however, been threatened by the rapid evolution of resistance in *Trichoplusia ni* (cabbage looper) populations. The spatial and temporal patterns of *Bt* resistance in *T. ni* greenhouse and field populations strongly suggest that resistant moths disperse from greenhouses treated extensively with *Bt* to 'unselected' neighbouring greenhouse populations early in the growing season. To quantify dispersal patterns, we have performed a genetic analysis using amplified fragment length polymorphism techniques. We will discuss the relationship of the patterns of *Bt* resistance to the genetic structure of cabbage loopers in greenhouse and field populations. This unique analysis of large scale patterns of *Bt* resistance and genetic population structure of a major Lepidopteran pest will allow informed decisions on resistance management in this system. In addition it will provide necessary empirical data for the formulation of predictive models of selection and resistance that may have application to other systems in which refuges are used as the basis of resistance management.

Contributed paper. Tuesday, 9:00. **68**

Effects of *Diabrotica*-resistant Cry3Bb1-Bt-maize on saprophagous Diptera and their coleopteran predators

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The Western Corn Root Worm (*Diabrotica v. virgifera*) first time occurred in Germany in July 2007. Four different maize cultivars including *Diabrotica*-resistant MON88017, were assessed in respect to its effects on saprophagous Diptera and predators out of Carabidae and Staphylinidae. The methodological approach comprised a hierarchic order of different ecological scale levels (agro-ecosystem, population, organisms). Abundance and species composition of both Diptera and their predators were recorded in the field. Most saprophagous Diptera belong to Sciaridae (fungus gnats), of which the predominant *Lycoriella castanescens* was used for feeding trials. It was tested whether mortality, pupation, hatching rates, duration of larval development and pupation were affected by uptake of Cry3Bb1-contminated plant tissues. Species of Carabidae and Staphylinidae were fed with Sciaridae-larvae reared on Bt- and non-Bt-maize-litter respectively. In a similar way *Diabrotica*-larvae were offered as prey. Toxin analyses of saprophagous Diptera and predators reared with Bt-plant parts or feeding on Bt-contaminated prey contained Bt-toxin up to 1.6% (decomposers) and 14.0% (predators) of the toxin level recorded in the source material. Predators collected from Bt-maize fields stated these findings. Thus, Bt-toxin is transferred into the food chain. Predators feeding on prey containing Cry3Bb1-toxin showed a significant delay in accepting the prey in comparison to prey free of Bt-toxin, but this didn't result in higher mortality or less longevity. However, predators which were fed with Sciaridae-larvae containing Bt-toxin produced significantly less offspring than those feeding on prey reared with non-Bt-maize litter. Thus, an uptake of Cry3Bb1-toxin by carnivorous beetles doesn't lead to a higher mortality, but results in subtle effects like lower fertility of the females.

Contributed paper. Tuesday, 9:15. **69**

Preliminary results on the interaction between *Bacillus thuringensis* and Red Palm Weevil

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The red palm weevil (RPW), *Rhynchophorus ferrugineus* (Olivier) causes significant damage to a wide variety of palm species. This pest originates from southern Asia and Melanesia, has been spreading westward since the 1980s. Recent detection of *R. ferrugineus* was reported from France, Greece, and Italy. Actually there is a strong emphasis on the development of integrated pest management based on biological control rather than on chemical insecticides. However the success of both the systems is often insufficient and RPW seems to be a pest very difficult to control. In this concern, it has been found advisable to investigate the natural defence of this curculionid with particular regard to its reaction to the entomopathogen *Bacillus thuringensis (Bt*). The *RPW* haemocytes, the main immunocompetent cells in insect, are described. RPW larvae of III and IV stage were feed with pabulum containing sub-lethal doses of commercial product of Bt register against Coleoptera. Bt was found in the hemolymph of the insect, showing the possibility for the bacterium to colonise RPW. We show also that the number of haemocytes is reduced in the RPW larvae feed with Bt. However an attempt to establish if these changes were significant was unsuccessful, as many larvae were still alive even after bloodshed for several days. However the results suggested that the study of immune system of the pest and its relationship with the potential pathogens are key factors to understand the high capacity of survival and infestation of the RPW and that could be helpful in the integrated pest management tools.

Contributed paper. Tuesday, 9:30. **70**

Genomics of the silkworm *Bombyx mori*: Tissue specificity and time course of gene expression in response to parasitization by tachinid flies

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The silkworm, *Bombyx mori* (Lepidoptera: Bombycidae) is an important insect with immense economic value and is also a model organism for research on Lepidoptera genomics and genetics. In sericulture, most of the damage results from pests and diseases. The tachinid fly, an endo-larval parasitoid of the silkworm, is often the most destructive among the pests. To understand the molecular mechanism of the host-parasitoid relationship between *Bombyx mori* and its tachinid flies, we identified by oligonucleotide microarrays, genes, which are upregulated or down regulated after parasitization by three tachinid species *Exorista japonica, Drino inconspicuoides* and *Pales pavida* that exhibit differing oviposition strategies. Variations in transcriptional profiles of several genes as well as patterns of co-expression between and among parasitoid species were observed. We further investigated gene expression in particular tissues and also examined their time course 0, 3, 5, 6 days after parasitization. Some of the up-regulated or down regulated genes were expressed widely in tissues; hemocytes, fat body, silkgland, midgut, malpigian tubules, testes, ovaries and central nervous system while others were restricted in range. Results also revealed that the timing of gene expression was variable. The study highlights the molecular mechanism of the host-parasitoid association between *B. mori* and tachinid flies.

CONTRIBUTED PAPERS Tuesday, 8:00-9:45

NEMATODES 2

Contributed paper. Tuesday, 8:00. **71**

Unravelling interspecific variability in virulence of four entomopathogenic nematodes to four white grub species: Virulence, infectivity, penetration sites

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Understanding the base for differences in entomopathogenic nematode (EPN) virulence to different white grub (WG) species may allow optimizing EPN use against these pests. WGs have coevolved with soil entomopathogens and developed behavioral, morphological, and physiological barriers to infection. We are using a standard set of WGs (*Popillia japonica*, *Anomala orientalis*, *Cyclocephala borealis*, *Rhizotrogus majalis*) and EPNs (*Heterorhabditis bacteriophora*, *H. zealandica*, *Steinernema glaseri*, *S. scarabaei*) to study difference in these defense mechanism. *S. scarabaei* is by far the most virulent species against *P. japonica*, *A. orientalis*, and *R. majalis*. But virulence did not differ significantly among EPN species against *C. borealis*. When larvae were exposed for 6–72 h to 1000 nematodes, larval mortality and nematode establishment rate, and occasionally speed of kill, showed the same pattern within nematode-white grub combinations. But no two nematodes or white grub species had the same pattern for all white grub species or nematode species, respectively. Using glue to block mouth and/or anus as penetration routes it was determined that the *Heterorhabditis* spp. had excellent cuticular penetration ability but may also penetrate through mouth and anus. The *Steinernema* spp. preferred to penetrate through the mouth but also penetrated through anus and cuticle.

Contributed paper. Tuesday, 8:15. **72 STU**

Diversity of nematodes parasitizing slugs in the United States of America and the United Kingdom.

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We conducted surveys of nematodes parasitizing slugs in the United States of America and United Kingdom in order to gather data regarding diversity and evolution of parasitism. We collected slugs from 70 USA and 30 UK sites. All slugs were dissected and examined for the presence of nematodes. Extracted nematodes were subjected to a combination of morphological and molecular (sequencing the 5' segment of the small subunit ribosomal RNA gene) methods to determine their identity. Results showed that 20.2% of UK slugs were parasitized by nematodes compared to 5.3% in the USA, indicating a significant association between sample site (UK/USA) and prevalence of nematode parasites (*P* value <0.01). Initial comparison of the UK and USA sites show a number of similarities and difference in the diversity of nematodes. In the UK, the predominant nematodes species were found to be *Angiostoma*, *Phasmarhabditis and Agfa* spp., whereas in the USA the majority of nematodes were found to be *Alloionema*, *Angiostoma* and *Agfa* spp. No native USA slugs were found to be parasitized by nematodes. A more complete comparison will be made once identification is complete and new species are described.

Contributed paper. Tuesday, 8:30. **73 STU**

Can endemic entomopathogenic nematode populations be used in conservation biological control of the annual bluegrass weevil (*Listronotus maculicollis*)?

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Entomopathogenic nematodes (EPNs) are present in the soils of most ecosystems, yet little is known about their field ecology, limiting their use in conservation biological control. We investigated the dynamics of endemic populations of EPNs over a three year period to determine their potential to regulate populations of the annual bluegrass weevil (*Listronotus maculicollis*) (ABW), a major pest of turfgrass. *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* were isolated from ABW cadavers at all sites and years, and to date are the only known natural enemies of the weevil. Endemic populations infected a wide range of instars, responded in density dependent fashion with nematode-susceptible weevil stages and caused moderate ABW generational mortality (up to 50%). However, EPN densities varied dramatically throughout the season in response to temperature and moisture extremes. Despite within season fluctuations EPNs displayed a distinct seasonal peak in abundance following peaks in ABW soil stages. The seasonal occurrence, sensitivity to environmental conditions and variable impact suggest that EPNs cannot reliably reduce ABW in a conservation biological control approach given the low aesthetic demands of turfgrass. The future prospects and barriers to using EPNs in conservation biological control are discussed.

Contributed paper. Tuesday, 8:45. **74 STU**

Development of a controlled release system for EPN application

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Several factors play a crucial role in determining the efficacy and persistence of *Steinernema feltiae* in controlling oilseed rape pests in the field. One of them is the application method. This study focused on developing a controlled release system (CRS), which would delay the EPN emergence after the application, and allow their gradual emergence. This would enable an earlier application of EPN at a time suitable for farmers, ensure protection of EPN from their antagonists, lower the application dose and the amount of water applied. Laboratory results showed that EPN emerge from the developed CRS to the soil with a delay of about 15 days, and continue to emerge gradually. The EPN in the CRS and in the soil remained infective throughout the experimental period, 35 days. The number of EPN within the CRS significantly negatively correlated and was associated with the number of EPN in the soil. In field experiments, CRS provided higher control of insect pests and a higher number of persisting EPN than the spraying method. Challenges for further studies include testing materials, which could be used in commercial production.

Contributed paper. Tuesday, 9:00. **75**

Formulation and application of entomopathogenic nematode infected cadavers for control of *Hoplia philanthus* in turf

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Entomopathogenic nematodes are commercially applied in aqueous suspension. These biocontrol agents may also be applied in nematode-infected insect cadavers. An attempt was made to formulate *Heterorhabditis bacteriophora* CLO 51 (Belgian strain)infected *Galleria mellonella* cadavers in wettable powders, *viz*. bentonite, diatomaceous earth, kaolin and fuller's earth. These formulations were tested for formulation consolidation, emergence of infective juveniles (IJs), and pathogenicity against *Hoplia philanthus* grubs in both pot and field conditions and compared with the efficacy of non-formulated cadavers. Of the four formulations tested, the kaolin-based formulation proved to be the most stable; kaolin adhered well to cadavers and did not cause suffocation to IJs. Emergence of *H. bacteriophora*IJs from cadavers formulated in kaolin (211,000 ± 40,533) was not significantly different from nematode emergence from non-formulated cadavers (177,080 ± 13,464). Freshly prepared cadavers of *H. bacteriophora*, 3-month-old cadavers and aqueous applications (50 and 75 IJs/cm^2) were applied to pots sown with ryegrass or turf field and infected with *H. philanthus*. Efficacy in grub control was assessed two weeks after nematode applications. In the pot experiment, freshly prepared cadavers of *H. bacteriophora*provided significantly higher grub control (58%) than 3-month-old cadavers (30%) or aqueous applications (38% and 42%). Similarly, under field conditions, lower but significantly higher grub control was achieved with freshly prepared cadavers (39%) than with 3-month-old cadavers (21%) or with aqueous applications (24% and 28%) of *H. bacteriophora* 2 weeks after application. However, after one year, cadaver application provided > 90% grub control; aqueous applications of *H. bacteriophora* only 55%.

Contributed paper. Tuesday, 9:15. **76**

Managing chickpea pod borer, *Helicoverpa armigera* (Hübner) with *Heterorhabitis indica*: A success story

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Studies on the effective management of chickpea pod borer, *Helecoverpa armigera* (Hüb) were carried out by integrating locally isolated entomopathogenic nematode, *Heterorhabditis indica* (RCR) with various entomopathogens and botanicals. The optimum dosage of infectives was standardized for third (LC50 of 145 IJs/larva) and fourth (LC50 of 195 IJs/larva) instars based on the concentration mortality response. Persistence study on chickpea foliage in field condition indicated that, infectives when sprayed along with 0.1 % glycerol survived better (80%) compared to other antidessicants. In compatibility studies, though the higher concentrations of aqueous leaf extracts of some selective botanicals were detrimental to nematode but were compatible at low concentrations. A series of laboratory bioassay was carried out to select best combinations of *H. indica* with other entomopathogens and botanicals against third and fourth instar *H. armigera* and these were tested in field for two consecutive years. Two year field evaluation indicated that, sequential application of *H. indica* + *Prosopis juliflora* (1 lakh IJs/l + 10%) at 50 and 75 days after sowing was superior with highest larval reduction (23.47%), minimum pod damage (11.27%) and maximum seed yield (19.24 q/h).

Contributed paper. Tuesday, 9:30. **77**

Potential for biocontrol of *Diaprepes abbreviatus* larvae in nurseries in southern California

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The Citrus Root Weevil *Diaprepes abbreviatus* is an invasive soil pest with an extremely broad host range which includes numerous agricultural and ornamental plant species. Native to the Caribbean region and an established pest in Florida, the weevil is currently the target of an eradication program in southern California. Commercial nurseries with *D. abbreviatus* infestations are subject to quarantine and significant financial losses as a result. We report the results of a study examining the efficacy of entomopathogens to control *D. abbreviatus* in a nursery environment. The entomopathogenic nematode, *Steinernema riobrave*, generated substantial levels of mortality and shows promise as a biocontrol agent against *D. abbreviatus* larvae in potted nursery plants.

CONTRIBUTED PAPERS Tuesday, 8:00-10:00

VIRUSES 2

Contributed paper. Tuesday, 8:00. **78**

Functional studies of *per os* infectivity factors of *Helicoverpa armigera* single nucleopolyhedrovirus

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In this manuscript, the *per os* infectivity factors (PIFs) of *Helicoverpa armigera* single nucleocapsid nucleopolyhedrovirus (HearNPV) were studied together. HearNPV bacmids with deletions of *p74* (*Ha20*), *pif1* (*Ha111*), *pif2* (*Ha132*) and *pif3* (*Ha98*) were constructed individually by homologous recombination in *E. coli* cells. Repaired bacmids with respective *pif*s were also constructed. Western blot analyses revealed that all four PIFs were structural components of the envelope of HearNPV occlusion-derived virus (ODV). Electron microscopy showed that the deletion of the *pif*s did not have any obvious affects to the morphology of the occlusion bodies. Bioassay analyses indicated that deletion of any of the above *pif*s resulted in loss of oral infectivity of OBs. The mixtures of the four *pif*-deletion mutants also resulted in the deficiency of oral infectivity, implying that the four PIFs must be structural components of the same ODV to accomplish their function. Repairing of the respective genes into the *pif*-deletion bacmids could rescue the oral infectivity of the *pif*-deletion viruses. Calcofluor which can damage the peritrophic membrane (PM) could not rescue the defects of the oral infectivity of the *pif*-deletion viruses, indicating PM is not likely to be the functional target of the PIFs.

Contributed paper. Tuesday, 8:15. **79 STU**

Influence of *pif* and *pif2* genes in the dynamics of recombinant insect virus populations

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A high prevalence of *per* os defective variants occurs among a Nicaraguan (Sf-NIC) population of the multiple nucleopolyhedrovirus of *Spodoptera frugiperda* (SfMNPV), indicating that interactions among genotypes are important for baculovirus survival. Past results have demonstrated a positive interaction between SfNIC-B (complete genotype) and SfNIC-C/D (*pif* defective genotypes) indicating that dilution of *pif* genes in the coinfected mixture resulted in increased infectivity compared to SfNIC-B alone. To confirm these results and evaluate the persistence of *pif* and *pif2* deleted genotypes in the population, two bacmid recombinants, SfNIC-BΔ16K (lacking the same 16.3 kb genomic region occurring in SfNIC-C) and SfNIC-BΔ*pifs* (encompassing a 2.8 Kb deletion including only both *pif* genes) were constructed, mixed individually with the complete genotype SfNIC-B and injected in *S. frugiperda* larvae at different ratios. After four successive passages in larvae genotype ratios converged to stable populations consisting of ca. 80%:20% of SfNIC-B:defective recombinants. This proportion resembles what is found in the wild-type SfNIC population. All mixtures re-established the infectivity of natural populations after they reached the equilibrium frequency and none of the deleted recombinant genotypes disappeared from the virus population. The proportion of *pif*-containing genotypes in the population seems to be critical for SfMNPV survival.

Contributed paper. Tuesday, 8:30. **80**

***Ac*MNPV *ac143* (*odv-e18*), a core gene that forms a cluster with *ac142*, is essential and mediates BV production**

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Autographa californica Multiple Nucleopolyhedrovirus (AcMNPV) *ac143(odv-e18)* is a late gene that encodes for a predicted 9.6 kDa structural protein that locates to the occlusion derived viral envelope and viral induced intranuclear microvesicles (Braunagel et al., 1996. Virology 122:100). To examine the role of *ac143*in the baculovirus life cycle, we used the AcMNPV bacmid system to generate an *ac143* knockout (KO) virus (AcBAC$^{ac142\text{REP-}ac143\text{KO}}$). Fluorescence and light microscopy showed that infection by AcBAC$^{ac142\text{REP-}ac143\text{KO}}$ is limited to a single-cell and titration assays confirmed that AcBAC$^{ac142\text{REP-}ac143\text{KO}}$ was unable to produce budded virus (BV). This is a very similar phenotype to the *ac142* KO virus. Progression to very late phases of the viral infection was evidenced by the development of occlusion bodies in the nuclei of transfected cells. This correlated with the fact that viral DNA replication was unaffected in AcBAC$^{ac142\text{REP-}ac143\text{KO}}$ -transfected cells. This study shows that *ac143* is essential, clusters with *ac142*, and is a baculovirus core gene.

Contributed paper. Tuesday, 8:45. **81 STU**

38K is a novel baculovirus nucleocapsid protein that interacts with other nucleocapsid proteins (VP1054, VP39 and VP80) and itself in *Autographa californica* multiple nucleopolyhedrovirus

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It has been shown that *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) *38K* (*ac98*) is required for nucleocapsid assembly. However, the exact role of 38K in nucleocapsid assembly remains unknown. In the present study polyclonal antibody against 38K were raised to investigate the relationship between 38K and the nucleocapsid. Western blotting revealed that 38K was expressed at the late phase of infection in AcMNPV-infected *Spodoptera frugiperda* cells and co-purified with budded virus (BV) and occlusion-derived virus (ODV). After fractionation of BV and ODV into nucleocapsid and envelope components, Western blotting showed that 38K was associated with nucleocapsids. Immunoelectron microscopic analysis revealed that 38K was specifically localized to nucleocapsids in infected cells and was randomly distributed over the nucleocapsid surface. Yeast two-hybrid assays were performed to examine potential interactions between 38K and 10 known nucleocapsid-shell-associated proteins (P78/83, PCNA, VP1054, FP25, VLF-1, VP39, BV/ODV-C42, VP80, P24, EXON0), 3 non nucleocapsid-shell-associated proteins (P6.9, PP31, ODV BV/ODV-E26), and itself. The results revealed that 38K interacted with nucleocapsid proteins VP1054, VP39, VP80 and 38K itself. These interactions were confirmed by coimmunoprecipition assays *in vivo*. These data demonstrate that 38K is a novel nucleocapsid protein and provide a rationale for why 38K is essential for nucleocapsid assembly.

Contributed paper. Tuesday, 9:00. **82**

The transmembrane domain of the AcMNPV GP64 protein plays specific roles in membrane fusion and virion budding

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The AcMNPV GP64 protein is important for cell receptor binding, membrane fusion, and virion budding. The GP64 transmembrane (TM) domain anchors the protein in the membrane but also specifically serves other roles. Replacing the GP64 TM domain with heterologous TM domains from other membrane proteins severely affects membrane fusion activity and virus infectivity. To examine the specific sequence requirements of the TM domain, we generated and analyzed a variety of mutations within the TM domain of AcMNPV GP64. Mutations included deletions, alanine scanning mutations, and single and multiple amino acid substitutions. We identified a critical TM domain length necessary for the membrane fusion function of GP64. All TM domain deletions resulted in reduced virion budding efficiency whereas deletions of the N- and C-terminal amino acids had variable effects on infectivity of the resulting virions. Analysis of amino acid substitutions and 3-alanine scanning mutations identified two regions (485-487 and 503-505) important for cell surface localization of GP64, and two regions (483-484 and 494-496) important for virus budding. Thus, in addition to the role of the TM domain in membrane anchoring, specific features of the hydrophobic TM domain play critical roles in membrane fusion, virus budding, and viral infectivity.

Contributed paper. Tuesday, 9:15. **83 STU**

The F-like protein of group I NPVs enhances the production and infectivity of the budded virus of *gp64*-null AcMNPV pseudotyped with the envelope fusion protein F of group II NPVs

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GP64 and F proteins were previously identified as the principle functional envelope fusion proteins in the *Baculoviridae*. The F-like proteins in group I NPVs, which use GP64 as a functional envelope fusion protein, are thought to be remnants of F in group I NPVs and lack a furin cleavage site. The F-like proteins were demonstrated to be a pathogenicity factor *in vivo*, but to be irrelevant for budded virus production in cell culture. However, in the current study we analyzed the function of the F-like protein Ac23 of *Autographa californica* MNPV in the absence of Ac-GP64 and found that the presence of Ac23 significantly enhanced infectious BV production of *gp64* null-AcMNPV pseudotyped with *Spodoptera exigua* MNPV F protein. Quantitative PCR further revealed the absence of *Ac23* leads to a lower production of infectious progeny virions as compared to control viruses. These findings provide evidence that the F-like protein not only enhances viral infectivity *in vivo*, but also *in vitro*. The role of the F-like protein as an auxiliary factor in BV maturation in group I NPVs will be further discussed.

Contributed paper. Tuesday, 9:30. **84 STU**

A highly conserved baculovirus gene *p48* is essential for BV production and ODV envelopment

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Autographa californica multiple nucleopolyhedrovirus (AcMNPV) *p48* (*ac103*) is a highly conserved baculovirus gene whose function is unknown. In present study, the role of P48 in baculovirus life cycle was investigated by generating a *p48* knockout virus via AcMNPV bacmid system. The resulting *p48*-null Bacmid $vAc^{P48\text{-}KO\text{-}PH\text{-}GFP}$ was unable to propagate in cell culture, while a 'repair' Bacmid $vAc^{P48\text{-}KO\text{-}PH\text{-}GFP}$ was able to replicate in a manner similar to a wild-type Bacmid $vAc^{PH\text{-}GFP}$. Titration assay and Western blot analysis confirmed that $vAc^{P48\text{-}KO\text{-}PH\text{-}GFP}$ was unable to produce budded viruses (BVs). qPCR analysis showed that *p48* deletion did not affect viral DNA replication. Electron Microscopy indicated that P48 was required for nucleocapsid envelopment to form occlusion-derived viruses (ODVs) and their subsequent occlusion. Confocal analysis showed that P48 prominently condensed in the center of the nucleus. Our results demonstrate that P48 plays an essential role in BV production and ODV envelopment in the AcMNPV life cycle.

Contributed paper. Tuesday, 9:45. **85 STU**

The baculovirus P10 protein and cellular microtubules are involved in the final stage of polyhedron formation

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The baculovirus p10 gene is evolutionarily conserved within the Alphabaculoviruses (lepidopteran nucleopolyhedroviruses) suggesting it plays an important role during infection of lepidopteran hosts. It has however been shown to be non-essential for virus replication in cell culture and caterpillar hosts. We have previously shown that the P10 protein is associated with the formation of two distinct cytoskeletal like structures; microtubule associated filaments and perinuclear tubules. P10 also shows a strong association with bundles of polyhedra that have been released from the host nucleus, which we have termed 'naked pol bundels'. We investigated the role of microtubules in the association of P10 with polyhedra by confocal microscopy and quantitative analysis and found that there was a significant difference in the level of P10-Pol association when infected cells were treated with the microtubule depolymerising drug Colchicine but not when treated with the microtubule stabilising drug Taxol. We observed that P10 had a stabilising effect on the microtubules abrogating the effect of the drugs. We further investigated early EM evidence that P10 plays a role in the association of the polyhedral envelope protein PP34 (AcORF-131, PEP) with the polyhedral envelope. Early indications are that deletion of p10 increases PP34 association with polyhedra.

SYMPOSIUM (Div. of Viruses) Tuesday, 10:30-12:30

Viruses of Bees

Symposium. Tuesday, 10:30. **86**

What could be the association of IAPV and CCD and protecting bees from IAPV

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Israeli acute paralysis virus (IAPV, refs 1. 2) has been found to be strongly associated with CCD (3). Segments of the IAPV genome have been shown to incorporate into the bee genome, and bees carrying integrated IAPV sequences become resistant to IAPV (2). We will discuss the postulated possibility (currently under study) that this integration may cause alteration in the bee behavior, leading to CCD. We will also show that Varroa is infected with IAPV, and the viral sequences are integrated into the Varroa genome. We will discuss the aspect that in addition to IAPV, stress is a factor that turn IAPV-carrying bees (in their genome) to develop CCD. We will also show that IAPV can easily and practically be silenced in bees and discuss the outcome of these experiments. 1. E. Maori, E. Tanne, I. Sela, *Virology* **362,** 342 (2007). 2. E. Maori *et al*., *J. Gen. Virol*. **88**, 3428 (2007). 3. D. Cox-Foster *et al*., *Science* **318**, 283 (2007).

Symposium. Tuesday, 11:00. **87**

The pitfalls of diagnosis interpretation in honey bee pathology: The case of deformed wing virus (DWV)

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The deformed wing virus (DWV) is one of the most prevalent virus in honey bee colonies. The high prevalence of DWV is likely correlated to its ability to be transmitted by the mite Varroa destructor. PCR amplification of DWV negative RNA strands in mites and the tremendous DWV loads recorded from mites argue for the replication of DWV in both varroa and bees. Besides, there is

strong evidence that DWV is also transmitted either horizontally by food exchange or vertically through eggs. DWV RNA loads measured in 360 seemingly healthy bee colonies from pools of 100 bees using quantitative PCR showed that bee colonies can tolerate very high loads of viruses without external clinical signs. We further identified DWV RNA in several bee organs by in situ hybridization and showed that queen and drone fertility could be impaired by such infection. In queen, the fat body cells were particularly infected while in drone, the whole reproductive reacted positively to DWV probe. Moreover, in crippled winged individuals from where very high DWV RNA genome copies were recorded, the digestive tract was heavily infected, indicating a probable negative effect on the digestive function. Our data strongly support that DWV produces pathogenic effects in severely infected individuals from the colony but these deleterious effects might not always have an impact on the colony fitness

Symposium. Tuesday, 11:30. **88**

Transmission and pathogenesis of DWV

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Deformed wing virus (DWV) is a viral pathogen of the European honeybee (*Apis mellifera*) associated with clinical symptoms and colony collapse when transmitted by the ectoparasitic mite *Varroa destructor* (*V. destructor*). In the absence of *V. destructor* DWV-infection does not result in visible symptoms. Analysis of the transmission routes for DWV revealed that DWV is transmitted horizontally within the colony and both, vertically and vectorially between and within colonies. Detailed analysis of horizontal and vertical transmission revealed that these routes did not cause any visible symptoms of disease suggesting that mite-independent transmission results in true covert infections. Overt DWV infections in bees are triggered by the transmission of the virus through the ectoparasitic mite *Varroa destructor*. Recently, it could be shown that replication of DWV in mites correlated with the occurrence of crippled wings. To further study this phenomenon viral loads were determined in mites. Correlation of these results with the development of clinical symptoms strengthened our hypothesis that replication of DWV in mites prior to transmission is one of the key factors in the pathogenesis of overt DWV-infections.

Symposium. Tuesday, 12:00. **89**

Host specificity of honey bee viruses and transmission routes: Implications for pollinator health

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RNA viruses are emerging as a serious threat to honey bee (*Apis mellifera*) health and are suspected as major contributors to the recent malady, Colony Collapse Disorder. Understanding the transmission of these viruses can shed valuable light on the epidemiology of this syndrome. In addition, the recent detection of Deformed Wing Virus in bumble bees as well as some of these viruses in in-hive food reserves of honey bees suggests a possible wider environmental spread of viruses with potential impact on the overall pollinator community. We studied the distribution of viruses in honey bees, their pollen loads and in other non-Apis hymenopteran pollinators collected from flowering plants. All the samples were analyzed with reverse transcriptase-PCR and virus identity was confirmed by sequencing. We report for the first time the molecular detection of picorna-like RNA viruses (deformed wing virus, sacbrood virus and blackened queen cell virus) in pollen pellets collected directly from forager bees. Furthermore, pollen pellets from some uninfected foragers were detected with virus, indicating a potential role of pollen in viral transmission. These viruses were found in eleven other species of native bees and wasps, expanding the known host range of these viruses and suggesting a possible deeper impact on the health of our ecosystem. Sequence comparisons of viruses isolated from honey bees, pollen and other non-Apis hymenopteran species indicate that the viruses are circulating freely among these species. In addition, the Israeli Acute Paralysis virus was detected in non-Apis pollinators near CCD apiaries but not in those near healthy non-CCD apiaries. Our findings increase the understanding of virus epidemiology and may help explain bee disease patterns and pollinator population decline.

Tuesday, 10:30-12:30

POSTERS – 1

BACTERIA

Poster / Bacteria. Tuesday, 10:30. **B-01**

Generation of a *Manduca sexta* larval midgut EST collection

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The Tobacco hornworm *Manduca sexta* is a common model organism widely used for biological experimentation, as they are easily raised under laboratory conditions, the larva are large and are relatively easy to dissect and isolate organs from. Despite its extensive use in biological fields like innate immunity, Bt delta-endotoxin (Cry toxins) mode of action, or plant-insect interaction studies, the genomic resources available for this organism are poor: 468 CDS and 3317 ESTs (none of them from midgut tissue) could be found in GenBank in April 2008. We attempted to improve this by constructing a larval midgut normalized cDNA library and by generating a dedicated EST collection. ESTs were obtained by classic Sanger sequencing but also by shotgun pyrosequencing using the 454 technology. EST data related to Cry toxin mode of action will be presented.

Poster / Bacteria. Tuesday, 10:30. **B-02**

Understanding the interactions of two novel Cyt-toxins

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Bacillus thuringiensis (Bt) Cyt genes encoding hemolytic and cytolytic toxins constitute a gene family, which are divided into two groups: Cyt1 and Cyt2. Within this family is Cyt2Ca, a 26 kDa protein protoxin which is cytolytic to a broad spectrum of insects in vitro. Within the same operon, there is a second gene encoding a protein exhibiting a high degree of similarity to Cyt2Ca, but no detectable insecticidal activity in vitro. Here we use Ligand Blot analysis and Planar Lipid Bilayer to gain insight into the overall MOA of these toxins and their possible interaction.

Poster / Bacteria. Tuesday, 10:30. **B-03**

Variability in the *cadherin* gene in the European corn borer, *Ostrinia nubilalis* (Hübner)

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The use of transgenic Bt-maize is increasing yearly (last year accounting for about 19% of the total maize planted area in the world) because of the efficient control of the corn borers, in especial *Ostrinia nubilalis* . Resistance to *Bacillus thuringiensis* (Bt) insecticidal toxins has been linked to the 12-domain *cadherin* locus in 3 lepidopteran species. The *O. nubilalis cadherin* gene has been revealed as a complex gene of about 20 kbp in length, with 34 introns. In the present work, we have studied the size polymorphism of the gene in a Spanish population, by amplifying the genomic sequence of the gene in 16 overlapping regions. The variability observed was not uniformly distributed, with a maximum in region 14 and a minimum (no polymorphism) in region 4. All this size variability must be due to changes in the intronic regions because we found no detectable size differences in mRNA. This variability can be useful to select appropriate polymorphic regions to be used as markers of this gene in experiments such us to determine the genetic linkage of the cadherin to Bt resistance traits.

Poster / Bacteria. Tuesday, 10:30. **B-04**

Comparison of wild-type and mutant forms of Bt toxin Cyt1A in molecular dynamics simulations

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Some mutations in the mosquitocidal toxin Cyt1A from Bacillus thuringiensis var. israelensis are known to abrogate the toxin's activity, while others do not. The loss of hemolytic or cytolytic activity is presumably due to changes in the toxin's structure and/or dynamics. We used molecular dynamics simulations to gain insight into the effect of three selected mutations. According to Ward et al. (J. Mol. Biol. 202, 527 (1988)), mutation K225A caused a loss of cytotoxicity and binding to lipid, while mutations K118A and K198A had no effect. We found that K225A mutation eliminated 3 hydrogen bonds of K225 (with L123, V126, and Y189), which resulted in disengagement of the alpha-helix hairpin C/D from the central beta sheet and in disruption of the latter. Simulations up to 10 ns showed that changes in mutant K225A, but not in K118A and K198A, spread throughout the protein. Free-energy calculations indicated that the inactive mutant K225A is significantly less stable (by 5 or 12 kcal/mol, respectively) than the still-active mutants K198A or K118A. Our results suggest that the mutant toxin is inactivated due to an overall change in conformation and diminished stability rather than due to a localized alteration of a "binding" or "active" site.

Poster / Bacteria. Tuesday, 10:30. **B-05**

Chitinase profiles and insecticidal effects of bacteria originated from hazelnut pests

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Chitinase is a very important virulence factor of pathogenic bacteria because of its ability to degrade insect' peritrophic matrix. In order to find chitinase producing bacteria, we screened our bacterial stock which contains 108 bacterial member derived from microbial flora of coleopteran group insect species responsible for serious damage on hazelnut. We determined chitinase producing bacteria, tested the chitinase producing ability of bacteria on the M9 chitin-agar and amplified the conserved domains of chitinase positive bacteria with specific primers. We also, tested the chitinase activities with chitin-agar diffusion and di-nitrosalicylic acid (DNS) methods among chitinase positive bacteria. Then, we compared these results with previous bioassay studies to determine the relationship between the chitinase activity and the insecticidal activity. 21,3% Coleoptera originated bacteria showed chitinase activity. Also we found a very strong relation between chitinase activity from chitin-agar diffusion method and insecticidal activity of isolates but di-nitrosalicylic acid method. In order to find and develop more effective agents in biological control for especially coleopteran insect pests, selecting chitinase-producing bacterial strains with chitin-agar diffusion method is very useful.

Poster / Bacteria. Tuesday, 10:30. **B-06 STU**

Interaction between REPAT members, a family of pathogen induced proteins

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In previous studies, *Spodoptera exigua* midgut gene expression was compared between larvae exposed and non-exposed to the *Bacillus thuringiensis* Cry1Ca toxin. A new gene family showing increased expression after exposure to different *B.thuringiensis* toxins and also after infection with baculovirus was identified. They were named REPAT (Response to Pathogen). No homology of these proteins was found in the public sequence database, and nothing was known about their function at molecular level. In order to study the possible function of REPAT1, we aimed to identify putative interactors using the yeast-two-hybrid technology with GAL4 system. First, Repat1 gene was cloned in a bait vector with a DNA binding domain and checked for autoactivation in yeast transformants. Next, a cDNA library from *S.exigua* midgut (*B. thuringiensis* exposed and non-exposed larvae) was obtained in the appropriate prey vector and used in the yeast two hybrid experiments for the screening of proteins interacting with REPAT1. Positive clones were obtained and identified by sequencing. REPAT4 was identified as REPAT1 interactor, as well as a new member of the REPAT family, REPAT5. Finally, a mating assay was carried out in order to confirm all the possible interactions between the different members of the REPAT family.

Poster / Bacteria. Tuesday, 10:30. **B-07**

Expression profiles of aminopeptidase genes in *Heliothis virescens* larvae exposed to Bt toxins

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A microarray containing 15,202 probes developed from Heliothis virescens expressed sequence tags (ESTs) were hybridized with labeled complementary RNA (cRNA) from midgut tissues of fourth instar larvae fed on a diet containing 1 ppm Cry1Ac for 0.5, 2, 6, and 24 hours. Larvae fed on a diet without Cry1Ac were used to obtain control cRNA. Expression levels of several aminopeptidases were investigated using normalized data.

Poster / Bacteria. Tuesday, 10:30. **B-08 STU**

Spodoptera exigua gene expression profile in response to sublethal intoxication by a commercial _Bacillus thuringiensis_ based product

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The beet armyworm *Spodoptera exigua* (Hübner) is a polyphagous insect pest that causes serious damage to numerous cultivated crops and it is widely distributed around the world. Nowadays, it is being controlled using different methodologies such as sex pheromones, chemical insecticides, cultural measures, and *Bacillus thuringiensis* products such as Xentari®. In the present work, in order to understand the mechanisms that are involved in physiological response to *B. thuringiensis* products, the transcriptional profile from the midgut of *S. exigua* larvae exposed and non-exposed to Xentari was determined. The experiments were performed using a cDNA macroarray, derived from a Suppression Subtractive Hybridization (**SSH**) library, which contained 588 unique ESTs. Comparison of the gene expression profile between the non-exposed and exposed larvae revealed that around 35% of the analysed ESTs were differentially expressed. Overall results showed that genes involved in metabolic function such as lipases or proteinases were generally down-regulated. In contrast, genes related to stress or response to pathogens were mostly up-regulated. Interestingly, among the up-regulated genes in the Xentari exposed insects, we found two ESTs coding for new members of the REPAT proteins family.

Poster / Bacteria. Tuesday, 10:30. **B-09**

Drosophila* embryos as a novel system for testing insecticidal toxins *in vivo

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Drosophila embryos are currently being developed as models of both wound repair, and phagocyte (hemocyte) behaviour. We have been micro-injecting toxins (Mcf1) from insecticidal bacteria (*Photorhabdus*) to look at their effects on hemocyte behaviour. Here we show that following injection of either purified Mcf1, or recombinant *E. coli* expressing the toxin, that embryonic hemocytes are rapidly and dramatically paralysed. We find that paralysis is abolished in dynamin mutants, suggesting that endocytosis of Mcf1 by the hemocytes is required for toxicity. We also present work attempting to dissect the genetic basis of Mcf1 mediated paralysis by observing embryos from different *Drosophila* mutants deficient in their small GTPases and additional essential components of the cytoskeleton. These results indicate the utility of *Drosophila* embryonic mutants in elucidating toxin mode of action.

Poster / Bacteria. Tuesday, 10:30. **B-10**

Study on Bt susceptibility and resistance mechanisms in the sugarcane borer, _Diatraea saccharalis_

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Rapid adoption of Bt corn applied heavy selection pressure on corn borers, especially the emerging sugarcane borer (*Diatraea saccharalis*) in Louisiana. By using novel F_2 screening technique, a Bt-resistant strain of sugarcane borer was developed in laboratory. The resistant borers are capable of completing larval development on commercial Cry1Ab corn. In this study, Cry1Ab-resistant and -susceptible strains of the sugarcane borer were subjected to Cry1Aa and Cry1Ac toxin treatments. Significant differences of larval mortality and growth were observed between the susceptible and the resistant strains. To understand the Bt resistance mechanisms in the sugarcane borer, midgut enzyme activities, including aminopeptidases, alkaline phosphatases, trypsins, chymotrypsins, esterases, and glutathione S-transferase, were examined *in vitro*.

Poster / Bacteria. Tuesday, 10:30. **B-11 STU**

Characterization of the _Heliothis virescens_ midgut regenerative response upon treatment with _Bacillus thuringiensis_ Cry1Ac toxin

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Cry1A toxins synthesized by the bacterium *Bacillus thuringiensis* target mature cells in the midgut of Lepidopteran larvae. In *Heliothis virescens*, Cry1Ac toxin causes mature midgut cell lysis, compromising epithelial integrity. To overcome this injury, midgut stem cells undergo quick cell divisions and differentiation to replace damaged mature cells. This process has been proposed to result in lower susceptibility and resistance to Cry1A toxins in some insect strains. In this work we utilize a combined genomic and proteomic approach to study this regenerative mechanism. Based on previous reports, our current hypothesis is that this process is regulated by growth factors and cytokines synthesized by dying mature midgut cells. We treated primary midgut cell cultures from *H. virescens* larvae with Cry1Ac or Cry3Aa (inactive against *H. virescens*), and isolated the proteins secreted by the dying cells. This secretome was then compared among treatments using proteomics and a bioactivity assay with stem cells to identify the growth factors involved in the regenerative process. This proteomic approach is coupled to a microarray analysis of changes in expression of putative growth factors in whole midgut from *H. virescens* larvae upon feeding on Cry1Ac toxin.

Poster / Bacteria. Tuesday, 10:30. **B-12**

High temperature could trigger rapid development of resistance to _Bt_ toxin Cry1Ac and deltamethrin in _Plutella xylostella_

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Human activities and the environment are greatly affected by climate and weather extremes. Various simulation models have predicted an increase of about 5°C in the July mean "heat index" over the

southeastern USA by the year 2050. It is well known how such changes will affect the dynamics of a number of insects however the impact on insect-protection tactics has received little attention. We explored the hypothesis that increased temperatures may enhance development of resistance to *Bacillus thuringiensis* toxin Cry1Ac and to pyrethroids or organophosphates. This could not only provide information of significant practical importance but an important generic model for bigger question relating to resistance, global warming and the effectiveness of GM technology. We selected a *Plutella xylostella* population collected from Serdang region (SERD4) at two different temperature 20ºC and 28ºC to investigate at which temperature resistance develops quickest. Our study showed that resistance to Cry1Ac and deltamethrin developed significantly more rapidly in sub-populations selected at 28ºC than at 20ºC. In contrast resistance to chlorpyrifos developed more rapidly at 20ºC.

Poster / Bacteria. Tuesday, 10:30. **B-13 STU**

Characterisation of novel resistance and cross-resistance to *Bacillus thuringiensis* crystal toxin

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The mechanisms of resistance to *Bacillus thuringiensis* crystal toxin were explored in the *Plutella xylostella* SERD4 population, which shows polygenic resistance to Cry1Ac with cross-resistance to the pyrethroid deltamethrin. Various immune parameters were screened including both cell-free and haemocyte-mediated responses. The composition of the intestinal microflora was compared between resistant and susceptible *P. xylostella* populations and eliminated to assess any contribution to Cry1Ac toxicity. The mechanism of cross-resistance to deltamethrin was also investigated. Esterase-mediated sequestration of Cry1Ac was tested using electrophoretic mobility shift assays and total esterase activity inhibition assays. Additionally, isozyme profiles and gut esterase activity were compared between various populations in an attempt to correlate the pattern and activity of carboxylesterases with cross-resistance.

Poster / Bacteria. Tuesday, 10:30. **B-14 STU**

Characterization of the Cry41Aa parasporin

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Owing to the reported cytocidal activities on human carcinoma cell lines, non-hemolytic parasporal proteins from *Bacillus thuringiensis* and related bacteria have been classed into a new family of proteins called Parasporins. The amino acid sequences of Cry41Aa1 & Cry41Ab1, grouped as Parasporin-3, and derived from the non-insecticidal Bt strain A- 1462 exhibit a remarkable similarity to the Bt insecticidal Cry proteins containing the typical three-domain structure. Currently, Parasporin-3 in the only reported parasporal protein that contains the 5 block regions conserved in Cry proteins. Besides bearing a typical three domain structure, it possess a HA-33 like domain from *Clostridium botulinum* that may impart cytocidal properties to the toxin. Parasporin-3 is found as the second gene (orf2) in a three gene operon, the third gene (orf3) resembles the 3' end of the larger Cry1A-type toxins. Parasporin-3 is therefore similar to several other 'split' toxins such as Cry5Ad, Cry30Aa, Cry19Aa and Cry10Aa. We have expressed the toxin in an E. coli system and have investigated the role of the three open reading frames in the expression of the parasporin toxin and have also invetigated the effect of removing the HA-33 like domain.

Poster / Bacteria. Tuesday, 10:30. **B-15 STU**

The efficacy of non-mosquitocidal Malaysian Bt isolates (Bt18) against three leukemic cell lines (CEM-SS, CCRF-SB and CCRF-HSB-2) and its mode of cell death

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Non-insecticidal *Bacillus thuringiensis* (Bt) parasporal proteins has caught the interest of researchers with its potential anti-cancer activity. The objective of this study is to determine the efficacy of parasporal proteins from non-mosquitocidal Malaysian Bt isolates (Bt18) against three leukemic cell lines (CEM-SS, CCRF-SB and CCRF-HSB-2). The solubilised and activated parasporal proteins of Bt18 exhibited anti-cancer potential by lowering the percentage cell viability of CEM-SS, CCRF-SB and CCRF-HSB-2 cells to 65%, 61.73% and 81.05%, whilst being non-cytotoxic to normal T lymphocytes at similar concentrations. Further purification of the parasporal proteins of Bt18 showed changes in inhibition selectivity, causing an increase in percentage cell viability for CEM-SS and CCRF-SB (77.18% and 69.13% respectively). However for CCRF-HSB-2 cells, viability was dropped to 54.6%. The Phosphatidylserine externalization assay, active caspase-3 assay and TUNEL assay detects and confirms apoptotic activity in leukemic cells treated with Bt18 parasporal proteins, while cell cycle analysis shows that there is cell cycle arrest in S phase of the treated leukemic cells. N-terminal sequencing of the upper and lower parasporal protein bands of Bt18 showed similarity with Cry 24Aa, 25Aa of Bt subsp. *jegathesan* and Cry 15Aa of Bt subsp. *israelensis*. Bt18 however does not share non selective hemolytic and cytotoxic characteristics as reported for Bt subsp. *Jegathesan* and Bt subsp. *israelensis* We suggest that Bt18 parasporal proteins share similar characteristics with Parasporins as it is non-hemolytic and non-cytotoxic towards normal T lymphocytes but inhibits cell viability of leukemic cells by cell cycle arrest and apoptosis.

Poster / Bacteria. Tuesday, 10:30. **B-16 STU**

Identification of GAPDH as a putative receptor for a 68-kDa *Bacillus thuringiensis* parasporal protein cytotoxic against leukaemic cells

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A Malaysian *Bacillus thuringiensis* isolate designated Bt18 expresses parasporal proteins specifically cytotoxic against CEM-SS, a leukaemic T lymphoblastoid cell (CD_{50}= 0.122 µg/ml) but does not harm normal T-lymphocytes. The separation of Bt18 parasporal proteins through anion exchange chromatography elucidated a 68-kDa parasporal protein which maintained specific cytotoxic activity against the leukaemic cell line albeit reduced potency. Polyclonal IgG (anti-Bt18) for the 68-kDa parasporal protein was successfully raised and purified. Toxin overlay blots using the anti-Bt18 IgG revealed that the 68-kDa parasporal protein bound to a 34-kDa protein from leukaemic T cell lysate. N-terminal amino acid sequencing of the 34-kDa protein was GKVKVGVNGFGRIG and NCBI protein BLAST search suggests the protein shares high sequence similarity with Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a protein with multiple functions, including a vital role in mitochondrial apoptosis.

Poster / Bacteria. Tuesday, 10:30. **B-17**

Different mechanisms of action of *Bacillus thuringiensis* Cry1Ac toxin along the midgut of lepidopteran larvae

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The general features of *Bacillus thuringiensis* Cry toxins mode of action in lepidopteran larvae have been clarified, but the molecular events that occur after ingestion and solubilization are not completely characterized. This characterization is pivotal to prevent and actively combat the development of resistance in insects exposed to pesticides based on *B. thuringiensis* products including GM crops. We have analyzed the effect of Cry1Ac toxin in terms of binding parameters and permeabilization capacity on brush border membrane vesicles (BBMV) prepared from the anterior and the posterior part of *Manduca sexta* and *Helicoverpa armigera* larval midgut. Cry1Ac bound specifically to BBMV from both larvae and no significant differences were detected in the binding parameters between the anterior and posterior regions within species. In contrast, in both species the permeabilization activity, measured by means of a voltage-sensitive dye, was significantly higher in the posterior region. We have also analyzed the inhibition of binding and pore formation by the sugar GalNac, a key residue in some membrane receptors. In the presence of GalNac, differences between anterior and posterior midgut regions and between species were detected.

Poster / Bacteria. Tuesday, 10:30. **B-18 STU**

Study of two midgut aminopeptidases from *Ostrinia nubilalis* Hübner

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Aminopeptidases N (APNs) have been identified as *Bacillus thuringiensis* endotoxins receptor candidates in several Lepidopteran species. Employing the RACE PCR technique we obtained two complete cDNAs corresponding to two APNs expressed in the midgut of *Ostrinia nubilalis* larvae. One of the sequences was 3624 bp long, and the predicted protein was composed by 940 aminoacids, whereas the other cDNA was 3226 nucleotides long, leading a putative protein composed by 994 aminoacids. The *in silico* study of the sequences, showed in both proteins a signal peptide, a GPI-anchor domain, a zinc-binding region $HEXXHX_{18}E$ and a GAMEN motif, characteristic of the gluzincin aminopeptidases. Moreover, several glicosilation sites were identified. The phylogram tree derived from ClustalW alignment grouped Lepidopteran APNs in five classes. The first sequence would belong to class 1 while the second one would belong to class 2. The expression of the two *O. nubilalis* APNs was studied during the larval development. Total RNA was purified from neonate larvae and from larvae 5, 10, 15 and 25 days old. RT-PCR reactions showed that both APNs where expressed during the whole larval growth.

Poster / Bacteria. Tuesday, 10:30. **B-19**

Characterization of the interactions of *Bacillus thuringiensis* delta-endotoxins with the gut of the pea aphid, *Acyrthosiphon pisum* (Harris)

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Hemipteran pests are not susceptible to the effects of known Bt toxins and have replaced the Lepidoptera as primary pests on Bt transgenic crops. Ideally, a strategy similar to the Bt transgenic plant technology could be applied for management of hemipteran pests. The objective of our current study is to delineate the physiological basis for the lack of insecticidal effect of the Bt toxins Cry1Ac and Cry3Aa on the pea aphid. We have shown that: (1) Both protoxins were stable in acidic buffers. (2) On treatment with cathepsin L, activated Cry1Ac was stable but Cry3Aa was digested to a single peptide of less than 20 kDa . (3) When incubated with membrane extracts from the pea aphid stomach, the Cry1Ac and Cry3Aa protoxins were hydrolyzed to a peptide with a molecular mass similar to that of the trypsin-activated toxins. This hydrolysis took 3 or 16 hr in the presence or absence of cysteine proteinase activators respectively. These results suggest that Cry protoxins are stable in the aphid foregut but could only be activated in the stomach of the pea aphid. The potential binding, oligomerization and insertion of Cry toxins into the aphid gut remain to be examined.

Poster / Bacteria. Tuesday, 10:30. **B-20 STU**

Analysis of receptor-binding region for effective improvement of Cry1Aa insecticidal activity

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As you know, Cry toxins produced by *B. thuringiensis* (BT) are widely used as safer alternatives to chemical insecticides. Although there are a few hypotheses on cry toxin's cell killing mechanism, all of those are the same in that insecticidal activity is attributed to receptor-binding. For acquisition of activity improved mutant in vitro, we established the method of screening mutants of high affinity for the receptor from the randomly-mutated toxin library by using phage-display system based on affinity maturation for receptor. To begin with, we analyzed the region which is crucial for receptor-binding to know which region we should induce mutation. For this, we prepared 13 cysteine mutant toxins and covered cysteine up specifically by small molecules. We investigated whether these molecules affected receptor binding ability of Cry1Aa as a steric hindrance and we speculated about the putative binding region on Cry1Aa toxin. Then, we constructed and screened libraries of Cry1Aa domain 2 random mutants of four amino- acids substitution in this region. Considering results from both cysteine mutants' binding inhibition assay and sequences of mutants after selection of mutant toxins using phage-display system, we will discuss about the receptor-binding region for effective improvement of cry toxin.

Poster / Bacteria. Tuesday, 10:30. **B-21**

Genetic stability of the putative marker *Bacillus thuringiensis* S76GFP$^+$ expressing a green fluorescence protein (GFP) in the absence of selective pressure

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Bacillus thuringiensis as bioinseticide has incited to significant knowledge on Cry proteins. However, the bacterium ecology remains poorly understood. Thus, a tractable *B. thuringiensis* developed for basic researches could help plant-microbe interactions studies, as well as, gene expression pattern in response to a particular environment. We constructed strain S76GFP$^+$ by electrotransferring a green fluorescence (*gfp*) gene expression vector (pAD43-25) to strain S76, a Brazilian wild type *B. thuringiensis kurstaki*, containing ca. eleven plasmids¾two of then bearing five *cry* genes. Fluorescence microscopy showed green streptobacillus, as early as, two hours after inoculation in liquid medium containing the pAD43-25 selection marker. Interestingly, although the *gfp* gene expression is constitutively regulated in vector pAD43-25, the Green Fluorescence Protein (GFP) could be detected throughout the entire cell cycle and, even, green free spores were noticed. Besides GFP synthesis, S76GFP$^+$ also maintained *cry* genes expression, as observed by SDS-PAGE. The present study revealed that after about 80 generations of S76GFP$^+$ cells grown on rich and sporulation media, with no selective pressure, were still able to maintain Cry proteins and GFP production, as accessed by SDS-PAGE and fluorescence, directly scanned, respectively. These results indicate that our marker *B. thuringisensis* is a useful tool to study the biology of this bacterium.

Poster / Bacteria. Tuesday, 10:30. **B-22 STU**

Construction of modified *Bacillus thuringiensis cry1Ac* genes based on *cry1-5* genes through multi site-directed mutagenesis

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Bt crystal proteins, encoded by *cry* genes, are a group of insecticidal proteins unique in the Gram-positive and spore-forming bacterium, *Bacillus thuringiensis*. These cry genes are widely applied as one of the most successful candidates for constructing transgenic plants resistant to pest insects. In our previous report, we found Cry1-5 had high insecticidal activity against *Spodoptera* larvae although its amino acid sequences showed high similarity (97.9%) to those of Cry1Ab which had low activity. In comparison with Cry1Ac, Cry1-5 had 12 different residues in domain I and II, and we focused on domain I and II regions and designed 10 mutagenic primers to change 12 residues. Through multi site-directed mutagenesis, we mutated the modified *cry1Ac* gene by plant codon usage in pOB-Mod-cry1Ac based on *cry1-5* and constructed 63 various mutant *cry* genes. In the further study, we will express those mutant proteins as a fusion form with polyhedrin using baculovirus expression system and subsequently do bioassay to *Spodoptera* larvae.

Poster / Bacteria. Tuesday, 10:30. **B-23**

Construction of a *Bacillus thuringiensis* engineered strain with high toxicity and broad insecticidal spectrum to Coleopteran by homologous recombination

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The larvae of Cockchafers are important insect pests in agriculture, horticulture, and forestry in both Europe and Asia. In China, *Anomala carpulenta* and *Holotrichia parallela* break out in the similar periods. But until recently, there were no literatures that reported the *B. thuringiensis* strains or *ICP* genes for control both *Anomala carpulenta* and *Holotrichia parallela*. A thermosensitive allele recombination system was developed to construct genetically modified *B. thuringiensis* strains encoding a crystal protein particularly active against Coleopteran species *Holotrichia parallela*. An integrative vector p8EC carrying *cry8Ca2* gene with high toxicity to *Anomala carpulenta* was constructed, and transformed into *B. thuringiensis* isolate 185. The *cry8Ca2* gene was integrated into the internal of transposons *Tnp167B* located on the endogenous plasmid of the host strain. Then the vector was eliminated by moving recombinant cultures to 38°C. Recombinant *B. thuringiensis* strains 185-F6 was obtained, and *cry8Ca2* gene was stably expressed in measurable amounts and did not reduce the expression of endogenous crystal protein genes. Bioassay results showed that 185-F6, in addition to the activity against *Holotrichia parallela* larvae present in the parental strains, exhibited a high level of activity against *Anomala carpulenta*.

Poster / Bacteria. Tuesday, 10:30. **B-24**

Engineered *Bacillus thuringiensis* 3A-HBF with insecticidal activity against Scarabaeidae and Chrysomelidae

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With the increasing environmental burden exerted by chemical pesticides, the need to develop alternative biocontrol pesticides becomes urgent. To broaden insecticidal spectrum of *Bacillus thuringiensis*, a important biocontrol agent, the recombinant plasmid pSTK-3A containing *cry3Aa7* was introduced into wild *B. thuringiensis* strain HBF-1, which contained *cry8Ca2* gene toxic to scarab larva *Anomala corpulenta*. Both Cry8C (130 kDa) and Cry3A (67 kDa) protein produced by the engineered Bt strain 3A-HBF was verified by SDS-PAGE and Western blot analysis. Flat rectangular crystals of Cry3Aa7 toxin protein and spherical crystals of Cry8Ca2 toxin protein were observed simultaneously under scanning electron microscope. The plasmid pSTK-3A showed high segregational stability when engineered strain 3A-HBF was grown in beef extract medium without antibiotic. 3A-HBF strain showed extra toxicity against Colorado potato beetle (*Leptinotarsa decemlineata*, CPB) besides *Anomala corpulenta*. The corrected mortality to CPB larvae was 100% after 24 hours, and to *A. corpulenta* was 100% two weeks later. This is the first report on engineered Bt strain which is insecticidal to two coleopteran pests,

including scarabaeidae and chrysomelidae. The results may offer a practical alternative for the two pests of Bt products in field application.

Poster / Bacteria. Tuesday, 10:30. **B-25**

Studies on protease-resistant core form of *Bacillus thuringiensis* Cry1Ie toxin

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cry1Ie genes were silent in *Bacillus thuringienesis* strains, but can be overexpressed in *Escherichia coli*, and the protein was toxic to *Plutella xylostella*, *Ostrinia furnacalis*, and soybean pod borer. A protease-resistant core form with a molecular weight of approximately 55 kDa that existed among the products of trypsin digestion was purified by Superdex-200 column. An oligomer and monomer of the protease-resistant core form were obtained during purification and collected separately. The oligomer comprises a small quantity of dimer and a large amount of higher aggregates larger than tetramers. The oligomer did not easily get reconverted into the monomeric form; while the monomer initially remained monomeric for a long time, but it was partially converted into an oligomer after a couple of days. It was determined that the N-terminal amino acid residue sequence of the purified protease-resistant core form of Cry1Ie started at amino acid residue 154 of full-length Cry1Ie. The LC_{50} of the full-length Cry1Ie protein and the monomer and oligomer of the protease-resistant core form against the diamondback moth (*Plutella xylostella*) were 13.94, 21.47, and 1425.42 µg / ml respectively.

Poster / Bacteria. Tuesday, 10:30. **B-26 STU**

20kb DNA: What is it doing in Bt crystals?

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There have been a number of publications and reports proposing a role for 20kb linear DNA fragments in the crystallization and activation of the toxin-containing inclusion bodies within *Bacillus thuringiensis*. We have studied the distribution and properties of this DNA from a number of different sources and suggest that this form of DNA is found ubiquitously in bacterial species and may not have a specific functional role in the formation or activation of Bt crystals. We will present data on our observations that this DNA can be found in both vegetative and sporulated stages of Bt and is present in both crystalliferous and acrystalliferous strains. This form of DNA is also present in *E. coli*. We have also tested the hypothesis that the DNA is not actually linear but contains circular chromosomal and plasmid DNA.

Poster / Bacteria. Tuesday, 10:30. **B-27**

Evidence of the involvement of the C-terminal portion of *Bacillus thuringiensis* Cry1Ac delta-endotoxin in crystallization

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Cry1Ac is one of the most studied *Bacillus thuringiensis* delta-endotoxins. Structurally, the latter has been divided in two domains: the N-terminal and the C-terminal portions. Although many studies concerned the biochemical and molecular characterization of the delta-endotoxin N-terminal portion, there are just few reports dealing with the study of the role of the C-terminal part. Hence, we engineered Cry1Ac delta-endotoxins modified in their N-terminal part and studied the effect of such modification on crystallization, toxicity and delta-endotoxin production. For such purpose, 4 point-mutation affected or deleted Cry1Ac delta-endotoxins, named Cry1Ac* and Cry1AcD respectively, were constructed. The latter could not form crystals when expressed in an acrystalliferous *B. thuringiensis* strain. However, when expressed in a crystalliferous one, these altered proteins were shown to interact by their C-terminal parts with the endogenous delta-endotoxins and co-crystallize with them forming atypical crystals observed by electronic microscopy. This co-crystallisation between the altered dela-endotoxins and the endogenous ones conducted to a decrease in delta-endotoxin production (28 %) by the corresponding recombinant *B. thuringiensis* strains. The ability of altered delta-endotoxins to co-crystallize with native ones could be exploited to promote the crystallisation of foreign proteins by fusing them with C-terminal part of Cry1A delta-endotoxins.

Poster / Bacteria. Tuesday, 10:30. **B-28**

***Bacillus thuringiensis* serovar *thompsoni* HD542 crystal proteins: Solubilization, activation, and insecticidal activity**

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Cry15Aa protein, produced by *Bacillus thuringiensis* serovar *thompsoni* HD542 in a crystal together with a 40 kDa accompanying protein is one of a small group of non-typical, less well-studied members of the Cry family of insecticidal proteins, and may provide an alternative for the more commonly used Cry proteins in insect pest management. In this paper we describe the characterization of the Cry15Aa and 40 kDa protein's biochemical and insecticidal properties and the mode of action. Both proteins were solubilized above pH10 in vitro. Incubation of solubilized crystal proteins with trypsin or insect midgut extracts rapidly processed the 40 kDa protein to fragments too small to be detected by SDS-PAGE, whereas the Cry15 protein yielded a stable product of approximately 30 kDa. Protein N-terminal sequencing showed that Cry15 processing occurs exclusively at the C-terminal end. Cry15 protein showed in vitro hemolytic activity, which was greatly enhanced by preincubation with trypsin or insect gut extract. Larvae of the lepidopteran insects *Manduca sexta*, *Cydia pomonella*, and *Pieris brassicae* were susceptible to crystals and pre-solubilization of the crystals enhanced activity to *P. brassicae.* Activity for all three species was enhanced by pre-incubation with trypsin. Larvae of *Helicoverpa armigera* and *Spodoptera exigua* were relatively insensitive to crystals and activity against these insects was not enhanced by prior solubilization or trypsin treatment. The 40 kDa crystal protein showed no activity in the insects tested, nor did its addition or co-expression in *E. coli* increase the activity of Cry15 in insecticidal and hemolytic assays**.**

Poster / Bacteria. Tuesday, 10:30. **B-29**

Characterization of environmental isolates of *Bacillus thuringiensis* from northeastern Poland harbouring *vip3A* gene homologues

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Various strains of *Bacillus thuringiensis* have been used effectively as biological insecticides due to their production of highly specific crystalline proteins, the so-called Cry or d-endotoxins. Recently, vegetative insecticidal proteins (VIPs) secreted during vegetative growth of certain *B. thuringiensis* strains have been described. As VIPs, particularly VIP3A, are known to be active against lepidopteran larvae, there is significant interest in identifying or developing strains with novel Cry and VIP combinations for applied use. To this end, the purpose of this study was to determine (i) the presence of *vip3A* homologues in *B. thuringiensis* collected in northeastern Poland; (ii) the correlation between the *vip3A* and *cry* genes contents, as well as the diversity in chromosomal DNA patterns; and in particular, (iii) the diversity of *vip3A*. Of 166 *B. thuringiensis* isolated from small wild mammals, soil, and milk products, 16 (~10%) harboured *vip3A* homologues with high levels of sequence conservation. These *vip3A*-positive isolates were shown to contain genes encoding known lepidopteran-active toxins, such as *cry1* (11 isolates), *cry2* (8 isolates), and *cry9* (2 isolates). Finally, PFGE analysis of DNA profiles demonstrated marked diversity among these isolate. As such, further studies are required to determine whether these isolates vary in toxicity against lepidopterans.

Poster / Bacteria. Tuesday, 10:30. **B-30**

Characterization of a novel Cry9Bb δ-endotoxin from *Bacillus thuringiensis*

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The *Bacillus thuringiensis* serovar *japonensis* strain S725 produces spherical crystals harboring a major protein of about 130 kDa. This protein showed immunoafinity and high level of N-terminal sequence identity with Cry9 delta-endotoxins. A *cry9*-like gene from Bt S725 was cloned, sequenced and expressed in Bt. The cloned gene sequence contains a 3492 bp ORF, which encodes a polypeptide of 1163 amino acids, with a predicted molecular mass of 131.4 kDa. The deduced amino acid sequence was unique and showed 73% identity with Cry9Ba. The novel d-endotoxin was assigned to a new subclass, Cry9Bb, by the Bt Toxin Nomenclature Committee. The 130 kDa Cry9Bb protein formed crystals and produced two fragments around 69 and 58 kDa upon trypsin activation. It exhibited activity against the lepidopterans *Manduca sexta* and *Anticarsia gemmatalis*. The biological effect of an amino acid residue substitution, A84P, was investigated. The LC_{50} for the Cry9Bb crystals against *M. sexta* neonate larvae was 6.84 µg/cm^2, while the LC_{50} for Cry9BbA84P crystals was 0.78 µg/cm^2. PCR screening revealed that, in addition to *cry9Bb*, Bt strain S725 also contains *cry1I* and *vip3* genes. Transcription analysis, using RT-PCR, showed that the *cry1I* gene was transcribed at T_2 and T_5 stages of sporulation.

Poster / Bacteria. Tuesday, 10:30. **B-31 STU**

Identification and cloning of novel *cry* genes from *Bacillus thuringiensis* strain Y41

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Four novel *cry* genes were cloned by PCR-RFLP method from *Bacillus thuringiensis* isolate Y41, which was isolated from Hainan Province. The toxins accumulating within the cells consisted of major proteins of 66 and 140 kDa and forming spherically shaped crystals. Compared the sequences of these fragments with known holotype *cry* genes, the result indicated that three of them are similar with *cry40Aa1*, *cry30Aa1*, and *cry19Aa1* respectively, and one of them is not distinct similar with any reported *cry* genes. All toxins have typical characteristic of delta-endotoxin and containing five homology blocks (1-5) which present in most *B. thuringiensis* delta-endotoxins. These four novel *cry* genes were deposited in GenBank and named by the *B. thuringiensis* delta-endotoxin nomenclature committee as *cry40Ca1*, *cry30Da1*, *cry52Aa1* and *cry53Aa1* respectively.

Poster / Bacteria. Tuesday, 10:30. **B-32 STU**

The characterization of novel Bt toxins

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Despite the large numbers of Bt toxins already discovered there remains the potential for the discovery of new toxins or the creation of variants with improved activities through traditional or directed evolution techniques. Although many techniques now exist for the directed evolution of new proteins the screening of variant toxins for improved activities remains a labour-intensive and resource heavy activity. Using the toxins Cry1Ac, Cry1Ah and Cry1Ie we have investigated the possibility of using an in vivo method for the selection of improved recombinants. We will describe the basis of this selection procedure and present the results of initial feasibility studies.

Poster / Bacteria. Tuesday, 10:30. **B-33 STU**

Identification of new *cry* genes of *Bacillus thuringiensis* through the use of a system of universal primers

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Based on the known *cry* gene sequences of *B. thuringiensis*, three pairs of primers were designed from the 5 conserved blocks found in the delta-endotoxin coding region. Designed primer pairs amplify the regions between blocks 1 and 5, 2 and 5, and 1 and 4, respectively. *In silico* analyses indicated that up to 96% of the known sequences can be amplified by one or more of these pairs. Their ability to detect new *cry* genes was tested when DNA from *B. thuringiensis* strains showing atypical crystal morphology was used as template. Some 175 strains recorded as "atypical" in the CINVESTAV-IPN (LBIT-series) collection log were further selected by phase contras microscopy, SDS-PAGE, and SEM analyses. After a systematic amplification and sequencing of amplicons obtained from 27 strains, 5 putative *cry* genes showed

highest identities between 25 and 43%; and 4 more between 63 and 69%, to known *cry* genes. Complete sequencing of new *cry* genes is in an advanced phase.

Poster / Bacteria. Tuesday, 10:30. **B-34**

Genetic diversity of *cry* gene sequences of *Bacillus thuringiensis* strains analyzed by denaturing gradient gel electrophoresis

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A new approach to the study of the diversity of natural microbial communities is to analyze PCR products generated with primers homologous to relatively conserved regions in the genome through denaturing gradient gel electrophoresis (DGGE). This methodology allows the separation of DNA molecules that differ by single bases and therefore has the potential to provide information about variations in target genes in bacterial populations in natural systems. In this study, we modify a two-step PCR-based approach. The strategy allowed us the amplification of unknown *Bacillus thuringiensis cry*-related sequences present in a single strain. In a first step we used a primer pair of the *cry* genes-specific PCR system, based on the degenerate primers (OL1 and OL5). The obtained amplicons were used in a second (semi-nested) PCR for DGGE, in which *cry* degenerate primers OL3GC and OL5 were used. The resulting products were separated after DGGE. Each stained band should correspond to a single *cry* gen. The DGGE assay developed here provides a rapid and reliable way to analyze the genetic diversity of *cry* genes present in a single strain of *B. thuringiensis* in pure cultures, as well as in environmental samples.

Poster / Bacteria. Tuesday, 10:30. **B-35**

Cyanogenesis in *Pseudomonas entomophila*: An entomopathogenic bacterium

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Pseudomonas entomophila was recently identified to be the only pseudomonad that naturally infects and induces lethality of *Drosophila melanogaster* (Vodovar *et al.*, PNAS 2005; 102 11414-19). Complete sequencing of the 5.9-Mb *P. entomophila* genome exposed potential virulence factors but experimental evidence for most of them is still lacking (Vodovar et al., Nature Biotech. 2006; 24 673-679). Cyanogenesis (eg. HCN production) has been demonstrated in a small number of bacterial species and is thought to contribute towards their pathogenicity. The presence of *hcnABC* gene cluster (encoding HCN synthase) in *P. entomophila* genome led us to test if *P. entomophila* produces HCN. HCN was measured in liquid cultures (70-80 μM) and on solid media (roughly 500 μM). In contrast to the wild type, a mutant strain (ΔGacA) does not produce any HCN in liquid culture but produces some HCN on solid media (roughly 100 μM). These data demonstrate that the GacS-GacA two component regulatory system affects in a positive manner HCN production in *P. entomophila*, though a second unknown regulator is implicated in HCN production under certain physiological conditions. In conclusion we demonstrate for the first time cyanide production by *P. entomophila* and we determine genetic factors that affect HCN production.

Poster / Bacteria. Tuesday, 10:30. **B-36**

***Bacillus thuringiensis*: Genetic diversity of Brazilian Lepidoptera specific isolates**

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The aim of this work was to genetically characterize 1073 isolates of *B. thuringiensis*, proceeding from three Brazilian collections, with main emphasis on the analysis of the *cry*1 genes. The oligonucleotide sequences were amplified and obtained amplicons for each subclass was evaluated the gene type per bacterial collection. As a result 55,7% of the isolates reacted to the primer Gral *cry*1, and the subclasses *cry*1Aa, *cry*1Ab, *cry*1Ac, *cry*1Ad, *cry*1Ae, *cry*1Af, *cry*1Ag, *cry*1Bf, *cry*1Ca and *cry*1Fa were detected in high percentages among the isolates varying from 43.4 to 54.9%. It was observed a homogeneous subclass distribution among the set of isolates from these collections, with greater percentage of isolates harboring the *cry*1Ab (42.12%) and lowest percentage for the *cry*1Db subclass (0.6%). The genetic variability of the analyzed bacterial collections seems to point that the Piracicaba and the Jaboticabal subset as the major source of promising isolates for the control of Lepidoptera pests. For the Sete Lagoas subset of isolates in which these subclass frequencies were considered low (bellow 20%) is was mostly observed the *cry*1B gene type present in 38.5% of the isolates.

Poster / Bacteria. Tuesday, 10:30. **B-37 STU**

Characterization of an endophytic *Bacillus thuringiensis* strain isolated from sugar cane

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The main characteristic of *Bacillus thuringiensis* (Bt) is the formation of protein crystals during their sporulation phase. It is the most commonly used bacterium in the biological control of insect larvae of agricultural pests and disease vectors. Endophytic bacteria are important due to their potential to be used in the control of insect larvae that feed on plants and/or live in their interior. Approximately 800 endophytic bacteria were isolated from sugar cane and there are stocked at the Laboratory of Microbial Genetics (ESALQ/USP-Brazil). Among them, 43 isolates were classified as *Bacillus* spp. by their colony morphology. Observation of a parasporal crystal by optical microscopy revealed that one of the isolates was *B. thuringiensis* (CTH31RzB4). This strain has been characterized by means of optical and electron microscopy, protein SDS-PAGE profile, *cry*, *cyt*, and *vip* gene content, and toxicity assays against Lepidoptera larvae.

Poster / Bacteria. Tuesday, 10:30. **B-38**

Electron-microscopic and genetic characterization of '*Rickettsiella tipulae'*, an intracellular bacterial pathogen of the crane fly, *Tipula paludosa*

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'Rickettsiella tipulae', a rickettsia-like intracellular bacterial pathogen of larvae of the crane fly, *Tipula paludosa* (Diptera: Tipulidae), has previously been characterized as both an independent species within the genus *Rickettsiella* and a synonym of its type species, *Rickettsiella popilliae*. Recently, the taxon *Rickettsiella* has been transferred from the *Alpha-proteobacteria* (order *Rickettsiales*) to the gamma-proteobacterial order *Legionellales*. Here we present the electron microscopic identification of this rickettsial pathogen together with the first DNA sequence information for *R. tipulae*. The results of our 16S rRNA gene-based phylogenetic analysis clearly demonstrate that the reorganization in the order *Legionellales* is justified for the pathotype *'Rickettsiella tipulae'*. However, the same data do not reveal a phylogenetic basis to consider *'R. tipulae'* an independent species, but instead give conclusive evidence substantiating its species level co-assignment with the more extensively investigated *Rickettsiella* pathotype *'melolonthae'*, i.e. a synonym of the species *R. popilliae*. These results have been confirmed by a complementary phylogenetic analysis employing a Multilocus Sequence Typing (MLST) approach. Moreover, comparison of 16S rRNA data from *'R. tipulae'* and *'R. melolonthae'* with those from an isopod-associated further *R. popilliae*-synonymized pathotype, *'Rickettsiella armadillidii'*, suggests that the latter might better be assigned to a different species.

Poster / Bacteria. Tuesday, 10:30. **B-39**

Functional analysis of nematocidal protein Cry6Aa2 from *Bacillus thuringiensis*

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The *cry6A* gene of *Bacillus thuringiensis* 96860-8 was cloned and expressed. Nucleotide sequences blast showed that the cloned *cry6A* gene is *cry6Aa2*. Bioassay results demonstrated that Cry6Aa2 had high toxicity against *Caenorhabditis elegans* , whose LC_{50} was 38.35ng/cm^2. Sequence analysis results indicates there exist proteolytic cleavage sites at aa11 and aa382 of Cry6Aa2, which may may play a role in proteolytic activation processing of Cry6A. Moreover, disulfide bonds of Cry6Aa2 may be involved in its toxicity. Bioassay results showed that the toxicty of mutants R11T and L382I, which lost N-terminal or C-terminal proteolytic cleavage site respectively, were reduced. The toxicity of double mutant R11T &L382I, which lost both N-terminal and C-terminal proteolytic cleavage site, was most significant lower than that of Cry6Aa2, R11T and L382I. The toxicity of single cysteine mutants C402G and C404G had no significant difference with that of Cry6Aa2. The toxicity of the double cysteine mutant C402G&C404G, whose LC_{50} was 21.45ng/cm^2, was most significant higher than that of Cry6Aa2. These results indicate for the first time that the mechanism of action of Cry6A against nematode involves solubilization and proteolytic activation processing. Thebreaking apart of disulfide bonds and N-terminal and C-terminal activation of Cry6A are essential in this two steps, respectively.

Poster / Bacteria. Tuesday, 10:30. **B-40**

Characterisation of two *Bacillus thuringiensis* subsp. *morrisoni* strains isolated from *Thaumetopoea pityocampa* Den. and Schiff., (Lep., Thaumetopoeidae)

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Bacillus thuringiensis is widely used for the microbial control agent of insect pests. Many thousands of *B. thuringiensis* strains have been isolated from environmental samples. In this study, two *B. thuringiensis* isolates obtained from *Thaumetopoea pityocampa* Den. and Schiff., (Lep., Thaumetopoeidae), the most harmful insect pest for pine species, were identified and characterized in terms of their electron microscopy, SDS-PAGE analysis, *cry* gene contents, H-serotype and insecticidal activities. The presence of Cry3 and Cry1 proteins was confirmed by observation of 65 and 130 kDa proteins by SDS-PAGE in Tp6 and Tp14 isolates, respectively. PCR analysis showed that Tp6 contains *cry3* gene and Tp14 isolate contains *cry1* and *cry2* genes. According to H-serotype results, these bacterial isolates were identified as *B. thuringiensis* subsp. *morrisoni* (H8a8b). Toxicity tests were performed against six insect species belong to Lepidoptera and Coleoptera groups. The highest insecticidal activity is 100% for Tp6 isolate on the larvae of *Agelastica alni* and *Leptinotarsa decemlineata* and 100% for Tp14 isolate on the larvae of *Malacosoma neustria*, respectively. Our results indicate that *B. thuringiensis* subsp. *morrisoni* strains, Tp6 and Tp14 isolates, may be valuable as biological control agent for coleopteran and lepidopteran pests.

Poster / Bacteria. Tuesday, 10:30. **B-41**

Characterization of *Bacillus thuringiensis* strain collections from Spain and evaluation of their insecticidal activity against *Ceratitis capitata*

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The Mediterranean fruit fly is one of the most devastating fruit pests worldwide, current control is mainly based on synthetic insecticides. The environmental impacts they produce, in addition to development of resistance justify the need to implement sustainable control alternatives. *Bacillus thuringiensis* Berliner (Bt) based products lead bioinsecticides market. They have been proven to be active against insects of many orders, including dipterans. However, no active strain against *Ceratitis capitata* Wiedemann has been described to date. In this study, a collection of 376 Bt strains has been established from samples collected in the Valencian Community (Spain). This collection was characterized by means of phase-contrast microscopy, SDS-PAGE and PCR to detect 20 groups of *cry* and *cyt* genes codifying for toxins active against lepidopteran, coleopteran, dipteran and nematode species. PCR analysis identified 10 combinations among selected genes, being more abundant those effective against lepidopterans, present in more than half of the strains. Protein electrophoresis revealed 39 different profiles that, in many cases, could be correlated with bacterial morphology and gene composition. Toxicity bioassays against *C. capitata* were carried out for all strains in the collection, recording maximum mortalities of 30%. Additionally, bioassays with isolates from other collections (509 strains) were performed, showing similar mortality levels.

Poster / Bacteria. Tuesday, 10:30. **B-42**

Susceptibility to *Bacillus thuringensis* of neonates and older larvae of *Tortrix viridiana* L. (Lepidoptera: Tortricidae) from a natural reserve

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Tortrix viridana L. (Lepidoptera, Tortricidae), the green oak leaf roller, is one of the most serious pest for oak in the Mediterranean areas. Recently out-breaks of this phytophagous were recorded in Natural Reserve in Sicily (Italy) where treatments are generally forbidden, but the commercial, social and environmental value of the wood in the forest needs to be preserved. Thus in particular case, it could be necessary the use of some biopesticides as *Bacillus thuringiensis* var. *kurstaki*. To optimize the dose, baseline susceptibility of a commercial formulation of Bt was determined for neonates and older larvae. The bioassay was carried out with of 5 different doses raised on leaf disks, and the data were analysed with Probit analysis. The differences in susceptibility of the different ages of the *T. viridiana* larvae were recorded. For neonates larvae the calculated DL50 was 0.63 mg/ml while the same doses had little effect on the older larvae, showing a clear decrease in susceptibility with age and larval growth. The implications of these data in controlling this pest in the natural reserve are discussed.

Poster / Bacteria. Tuesday, 10:30. **B-43**

***Bacillus thuringiensis* as a biological control agent for the red palm weevil, *Rhynchophorous ferrugineus* (Oliv.) (Coleoptera, Curculionidae)**

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The red palm weevil (RPW), *Rhynchophorous ferrugineus* (Oliv.) (Coleoptera, Curculionidae) is an important pest of palm trees. In Italy was accidentally introduced in 2004 and in less than 3 years has became a tremendous problems for ornamental palms belonging to the genus *Phoenix*. Since chemical applications are difficult in urban areas, biological control methods should be preferred. A commercial preparation of the microbial entomopathogen, *Bacillus thuringiensis* subsp. *kurstaki pathotype H- 3A, 3b* registered against Coleopetera, was evaluated for biological activity against the RPW. Based on oral bioassays was considered the effective in controlling adults and mature larvae. Bioassays with diet contaminated by spores and δ-endotoxin of the bacterium indicated that adults were susceptible to infection. However the concentration of the product to be efficacy was rather high. The mortality of mature larvae exposed to treated diet was negligible, even if the effects on larval behaviour (reduction in feeding and movement) were observed. The potential use of *Bt* in controlling RPW is discussed as, at least with this strain, the degree of control achieved might be inadequate.

Poster / Bacteria. Tuesday, 10:30. **B-44**

New strategy for isolating novel nematicidal crystal protein genes from *Bacillus thuringiensis* strain YBT-1518

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This work describes a novel strategy for cloning *cry* genes from *Bacillus thuringiensis* by constructing library with *B. thuringiensis* as host and shuttle vector pHT304 as cloning vector and then screening by checking the formation of crystals. *B. thuringiensis* strain YBT-1518 shows toxicity against root-knot nematode and produces 54kDa and 45kDa crystal proteins. Eight out of three hundred colonies were found to produce crystals and its crystal proteins showed toxicity to nematode, *Meloidogyne hapla*. Seven colonies formed the same rice-shaped crystal as strain YBT-1518 does, while the other one did typical bipyramidal crystal. The rice-shaped crystals consisted of either 54kDa protein or 45kDa protein, while the crystal protein of the bipyramidal crystal was estimated 140kDa. The 45kDa crystal protein is encoded by a novel gene, formly *cry55Aa1*, which has not any significant homology to any *cry* genes. The 54kDa protein was encoded by *cry6Aa2*. Surprisingly, the 140kDa protein was the product of gene *cry5Ba2*. There is neither this 140kDa protein in the crystal protein contents nor the bypyramidal crystal in the sporulation culture. The gene cloning strategy described in this work provides a novel way to isolate novel and/or silent crystal protein genes from *B. thuringiensis*.

Poster / Bacteria. Tuesday, 10:30. **B-45**

Physiological characterization of accumulated poly-β-hydroxybutyrate(PHB) in *Bacillus thuringiensis*

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Poly-β-hydroxybutyrates(PHBs) are accumulated during exponential growth and then utilized very fast during the early stationary phase in *Bacullus thuringiensis* which produces spore and synthesizes insecticide proteins in the late life. PHB is a very important reserve material as carbon and energy when it produces a spore and synthesizes insecticide proteins, it is little known about PHB metabolized how to affect them in *Bacillus thuringiensis*. We addressed those questions by knockouted gene *pha*C, the key gene of accumulation of PHB, by inserted an erythromycin-resistance gene to replace gene *pha*C in *Bacillus thuringiensis* obtained PHB-negative mutant in this study. PHB-negative mutant of *Bacillus thuringiensis* was unable to synthesize PHB and its generation time was longer than the parent strain on Luria-Bertani medium. The ability of vegetative cell and spore of parent strain against UV irradiation and heat was much greater than the mutant strain. Physiological studies showed that the PHB-negative mutant strain excreted more formate, lactate, acetate, pyruvate, β-hydroxybutyrate, fumarate, malate citric acid and glutamine than the parent strain. The NAD^+/NADH and $NADP^+$/NDDPH ratio in the PHB-negative mutant strain was lower than that in the parent strain. When we fermented the parent strain and the mutant strain, the latter produce much less spore and can synthesize insecticide proteins but not form crystal. From those results, we can conclude that the accumulated PHB is important to *Bacillus thuringiensis* significantly when it forms spore and synthesizes crystal insecticide protein.

Poster / Bacteria. Tuesday, 10:30. **B-46**

Influence of different strategies of European corn borer (*Ostrinia nubilalis* Hübner) control on the content of contaminants in maize.

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The control of European corn borer (ECB) plays the most important in the prevention of mycotoxin accumulation in maize. Except of this, the occurrence of insecticide residues must be studied if chemical control used. Bt-maize, insecticides, and Trichogramma wasp were tested against ECB during period 2002-2007. To this purpose Bt-maize ('MON 810') and non-Bt hybrid ('Monumental') were used. The content of mycotoxins (NIV, DON, ADONs, T-2, HT-2, FUS-X and ZEA) in maize grain was evaluated. In addition, the incidence of insecticide residues (metoxyfenozide, indoxacarb) used in ECB chemical control of sweet maize was analysed. In Bt maize the lower occurence of toxinogenic micromycetes was observed. From the fungi linked to injuries by ECB to the most frequent species belonged: *Fusarium subglutinans, F. verticillioides, F. proliferatum, F. sporotrichioides*. Generally, the most frequent mycotoxin found in product was DON. NIV and ZEA appeared in some seasons only and the content of other mycotoxins was mostly bellow LOQ. A slight contamination by residues was observed in ear coats sampled during vegetation season. Before the harvest the metoxyfenozide residues was detected whereas indoxacarb declined better. Negligible contents (<MRL) of pesticide residues left by the tested insecticides were found in harvested sweet maize grain.

Poster / Bacteria. Tuesday, 10:30. **B-47**

Efficacy of different strategies of European corn borer (*Ostrinia nubilalis* Hübner) control in maize

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The effective control of European corn borer (ECB) is a very important aspect in food safety programmes in maize growing systems. Making holes in the stalks and in ears the pest larvae are a primary cause of maize infections by toxinogenic micromycetes dangerous both for human consumers and animals. The efficacy of selective insecticides (metoxyfenozide, indoxacarb), *Bt* maize and *Trichogramma* wasp (*Tw*) as preparation Trichocap ® against ECB was evaluated on several different locations during period 2002-2007. Before the harvest the plant injuries (tunnels and stalk breakage) caused by ECB were evaluated. The highest biological efficacy was achieved in Bt- maize (100%) in all experimental years. The effect of insecticide treatments was very good ranging from 80% to 95%. *Tw* applications resulted in satisfactory effect (cca 50%) in the most of years. The exception was previous season (2007) when the efficacy nearly 80% was observed. In addition, the concept of an antiresistant strategy in ECB control is proposed. In this concept *Tw* and selective insecticides can be applied in refugees used in insect resistant management in the protection against European corn borer. *The work was funded by the project No 1B53043 of the Ministry of Agriculture of the Czech Republic.*

Poster / Bacteria. Tuesday, 10:30. **B-48**

Identification of commercial BT-strains by molecular markers

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Bacillus thuringiensis is nowadays the most important biopesticide in the world. The ability of identifying a specific commercial strain of this micro-organism is crucial to assess its persistence in the environment. Several Authors described some PCR-based approaches as useful and rapid methods to face the problem. Following the Arbitrary Primer-PCR method proposed by Brousseau and Collaborators, we tried to recognize some different commercial strains of *Bacillus thuringiensis*. The three different primers indicated by the Authors allowed the clear separation of three out five of these strains.

Poster / Bacteria. Tuesday, 10:30. **B-49**

Host plant preference of spider mites on Bt-expressing and control potatoes

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A two-choice disc test was used to examine the effect of Cry3A expression on host-plant preference. Discrimination between a transgenic potato, *Solanum tuberosum* (Solanaceae) cv. Superior NewLeaf (Monsanto, USA) capable of synthesizing *Bacillus thuringiensis* toxin, and an isogenic cultivar was studied using the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae) under laboratory conditions. Adult females of spider mites were individually placed on leaf discs (one half transgenic and one half control) and observed at regular intervals. In addition, the distribution of *T. urticae* eggs on the discs was recorded. *T. urticae* females were found more frequently on control leaves than on transgenic leaves. The distribution of spider mite eggs reflected the observed biased distribution of females. These results indicate that potatoes expressing Bt for resistance against Colorado potato beetle are less preferred by spider mites under a choice test condition using excised leaves.

Poster / Bacteria. Tuesday, 10:30. **B-50**

Interactions between Cry1Ac, Cry2Ab, and Cry1Fa *Bacillus thuringiensis* toxins in the cotton pests *Helicoverpa armigera* (Hübner) and *Earias insulana* (Boisduval)

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Insect resistance to *Bt*-plants can be delayed by concurrent expression of several toxins in the same plant. New generation *Bt*-cotton, such as Bollgard II and WideStrike, simultaneously express two different Cry toxins, Cry1Ac and Cry2Ab, and Cry1Ac and Cry1Fa, respectively. The individual and combined toxic effects of Cry1Ac, Cry2Ab and Cry1Fa was determined in the cotton pests *Helicoverpa armigera* and *Earias insulana*, as were the interactions between these toxins. Singly, all three assayed toxins were more toxic against *E. insulana* than against *H. armigera*. Toxin Cry1Ac was significantly more toxic than the other two on *H. armigera*, while toxin Cry1Fa was the least toxic and caused no significant mortality. When combined, Cry1Ac and Cry1Fa showed an additive interaction in all proportions analyzed for both pests, whereas Cry1Ac and Cry2Ab interacted synergistically in all mixtures against *H. armigera*. In *E. insulana*, there was no synergism between

Cry1Ac and Cry2Ab but both these toxins showed a high insecticidal activity when administered individually and in mixtures. This study suggest that each particular toxin or toxin combination expressed in transgenic *Bt* cotton should be carefully selected depending on the most important pest species present in each geographical area.

Poster / Bacteria. Wednesday, 10:30. **B-51**

Development of the proteinaceous insecticide from a soil bacterium (*Bacillus thuringiensis)* using phage display

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Bacillus thuringiensis (Bt) produces the Cry toxin, the insecticidal protein which is induced production at the time of sporulation. This Cry toxin is safety for both human and animal but active only to some specific insects and widely used as the microbial-insecticide. However, it is fairly difficult to screen and discover a new Bt-strain with expected insecticidal specificity and activity from the nature. Contrary, it is theoretically possible to make activity improved toxin by increasing affinity to the receptor altering amino-acid sequences especially of the receptor-binding region of the toxin. In this research, we used a phage display system, which was developed in our laboratory, and tried to achieve the directed evolution of Cry toxins to increase the affinity to the receptor.

Poster / Bacteria. Tuesday, 10:30. **B-52**

Screening for more toxic δ-endotoxins of *Bacillus thuringiensis* for the management of *Spodoptera litura* in India

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Spodoptera litura is a major pest attacking important commercial crops like cotton in India. Commercial Bt cotton hybrids carrying Cry1Ac toxin (Bollgard I type) or Cry1Ac and Cry2Ab toxins (Bollgard II type) give adequate control of the target insect *Helicoverpa armigera*, however there is a common opinion among farmers that *S. litura* is an emerging problem in Bt cotton. There is an urgent need to screen for more toxic holotype and/or hybrid Cry proteins against *S. litura* to minimise the use of chemical insecticides in Bt cotton, the main objective of transgenic cotton technology. In laboratory screening experiments G27, the EEC hybrid toxin producing strain, was more toxic than other holotypes (1Ca and 1Fa) and hybrids (AbAbC and AcAcC) tested.

FUNGI

Poster / Fungus. Tuesday, 10:30. **F-01**

Differential UV tolerance amongst spore-cell types of the entomopathogenic fungus *Beauveria* (*Cordyceps*) *bassiana*

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The entomopathogenic fungus *Beauveria bassiana* is negatively affected by abiotic stresses such as heat and UV-B in solar radiation. Large variations among *B. bassiana* strains in tolerances to UV-B radiation have been demonstrated, but little is known concerning the underlying mechanisms involved. Hypotheses that may explain fungal strain differences in tolerance to the UV-B segment of solar radiation include: (a) variations in spore coat compounds, (b) internal accumulation of protective compounds, and/or (c) variations in intrinsic DNA repair mechanisms. In addition to thick-walled aerial conidia, with specific nutrient conditions and agitated liquid culture, *B. bassiana* produces two different single-cell types: blastospores that lack rigid cell walls, and thin-walled submerged conidia. In this study, we examined the UV resistance of aerial conidia, blastospores, and submerged conidia from four *B. bassiana* strains that displayed relatively low, medium, or high UV-B tolerance as aerial conidia. Aerial conidia were produced on potato dextrose agar, blastospores in Sabouraud dextrose broth, and submerged conidia in a fructose-based minimal-medium broth. Yeast extract was added to all three media (0.5%, 0.5% and 0.005%, respectively). The largest variation in UV-tolerance was with blastospores. Two strains, one with high and one with medium aerial-conidia UV-B tolerance, were highly UV-B tolerant as blastospores; whereas blastospores from two different strains, one with medium-level and one with low-level UV-B-tolerance as aerial-conidia, were highly susceptible to UV-B. In contrast, submerged conidia of all four isolates were highly UV-B tolerant, irrespective of the susceptibility of their aerial conidia and/or blastospores. These data suggest that UV-B tolerance probably is not mediated by variations in spore-coat thickness. Differences in intrinsic DNA repair mechanisms or internal accumulation of protective chemicals were not investigated. Submerged conidia, because of their consistently high UV-B tolerance, may be useful for biological control applications under high UV exposure conditions.

Poster / Fungus. Tuesday, 10:30. **F-02 STU**

Effects of *Beauveria bassiana* on the bark beetle *Ips sexdentatus* and on its predator *Thanasimus formicarius*

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Bark beetles are a major thread to the forest economy. Pathogens, such as *Beauveria bassiana*, could be promising candidates for their control. Before using *Beauveria bassiana* in the field, a lot of parameters have to be checked in the laboratory: Such as the effects on the bark beetles, and "side effects" on non targets. Therefore, the main intention of this study was to test the effects of *B. bassiana* on the bark beetle *Ips sexdentatus* and its predator *Thanasimus formicarius*. Infection experiments were conducted in the lab at 20°C and long day conditions. *B. bassiana* was isolated from *Ips typographus* and grown on malt extract agar. *I. sexdentatus* was collected from infested pine log sections and *T. formicarius* from pheromone baited traps. Adult *I. sexdentatus* and *T. formicarius* were inoculated with different concentrations of conidia suspension or by stripping off dry conidia from bark beetle cadavers. The experiments showed that *B. bassiana* killed a high percentage of *I. sexdentatus* (up to 100%) in less than 7 days, whereas the percentage of dead *T. formicarius* was remarkably low (less than 30% with highest spore concentrations).Thus these results provide a good base for further semi-field and field experiments.

Poster / Fungus. Tuesday, 10:30. **F-03**

Pathogenicity of *Beauveria bassiana* and *Beauveria brongniartii* to the bark beetle *Ips typographus* L. (Coleoptera: Scolitidae)

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The spruce bark beetle, *Ips typographus* L. (*Coleoptera: Scolitidae*) is the most important insect pest for oriental spruce trees (*Picea orientalis* Link) in Georgia. 2005-2006 at populations of *I. typographus* the entomopathogenic fungi - *Beauveria bassiana* and *Beauveria brongniartii* have been detected. *B. bassiana* (Georgian strain) and *B. brongniartii* (Germany strain) were tested to adult beetles of this pest. Inoculums of both fungi were obtained from originally isolates of the homologous host of *I. typographus.* Isolated fungi were cultivated on different media, i.e. MEA, PDA and BSM for 12-15 days at 25°C. Healthy bark beetles were collected by hand or cutting infested log section from the spruce trees and placed on spruce-bark pieces (10x10 cm) treated with of fresh cultural suspensions of *B. bassiana* and *B. brongniartii* (3.2 X 10^5 and 3.2 X 10^6 conidia/ml). The beetles of each variant were incubated with some spruce bark pieces at 20^o, 25^o, and 30^o C, without light and at 90% relative humidity. Mortality was recorded daily till the death of the last beetle by Abbot' formula (Abbot, 1925). Mortality of insect by action of of *B. bassiana* suspension was 79,5- 91.2% and *B. brongniartii* 33,3-45%.

Poster / Fungus. Tuesday, 10:30. **F-04**

Scanning the virulence of sixty isolates of *Beauveria* spp. and *Engyodontium albus* to the cattle tick *Boophilus microplus*

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The control of the cattle tick *Boophilus microplus* (Acari: Ixodidae) is cause for concern to the Brazilian cattle-raising industry. The current study evaluates the virulence of 60 fungal isolates, including five species of *Beauveria* and one species of *Engyodontium albus* (=*Beauveria alba*), that originated from several geographic regions, arthropod hosts or substrates. Aliquots of 50 mg of *B. microplus* eggs (~1000) were placed in test tubes and incubated at 27 ± 1ºC. Ten days after total hatch, larvae were immersed in aqueous conidial suspension (Tween 80 0.001%) at 10^5, 10^6, 10^7 or 10^8 conidia ml^{-1} for 1 minute, and larva mortality was recorded at 5-day intervals. Ten tubes of larvae were inoculated with each conidial concentration or control solution (no conidia). All 60 isolates of *Beauveria* spp. and *E. albus* isolates presented great variability in virulence to *B. microplus* larvae. The most virulent isolates were *B. bassiana* CG206 and CG464, which caused more than 90% mean mortality. In contrast, isolates UFPE479, UFPE496 and CG234 caused no larval mortality. The *E. albus* isolate and those *Beauveria* isolates other than *B. bassiana* presented low virulence against *B. microplus* larvae, with the exception of a *B. amorpha* (ARSEF4755) that caused mean mortality around 60%. Five of the *B. bassiana* isolates were chosen to be re-tested using *B. microplus* larvae from a geographically different population. All five isolates (Bb23, Bb44, ESALQ986, ESALQ747 and CG480) induced mortality faster than was observed with the first tick population. In conclusion, the present study has identified isolates of *B. bassiana* with high virulence against *B. microplus* larvae. Furthermore, the results indicate that different populations of this tick species may present different levels of susceptibility to *B. bassiana* infection. Thus, not only the genetic and physiologic conditions of fungal isolates, but also the susceptibility of *B. microplus* population may affect biological control efficacy.

Poster / Fungus. Tuesday, 10:30. **F-05**

The first record of *Beauveria bassiana* (Deuteromycetes) on the hibernating pupae of *Cameraria ohridella* (Lepidoptera: Gracillariidae)

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Cameraria ohridella Deschka et Dimic, is an important invasive pest of *Aesculus hippocastanum* in Europe. Present methods of its control are based on application of non-selective insecticides and composting or burning of leaf litter. Our project is aimed at entomopathogenic fungi as potential candidates for biological control of this pest. In the present work we report the first record of *Beauveria bassiana* (Bals.) Vuill. as an entomopathogenic fungus of *C. ohridella*. *B. bassiana* was found and isolated from hibernating pupae of *C. ohridella.* Samples of the horse chestnut leaves with diapausing *C. ohridella* pupae were collected in autumn 2007 at Ceske Budejovice, South Bohemia, the Czech Republic. The leaves were dissected and the pupae were individually placed into the 23°C and 95% R.H. Development of mycosis on pupae was frequently observed but only a few pupae were covered by mycelia with conidiophores and conidia of *B. bassiana.* The strains of the fungus were isolated from individual infected pupae and deposited in CCEFO (Culture Collection of Entomopathogenic Fungi Olesna) in the Czech Republic. The virulence of the isolated strains is being evaluated. This work was supported by the MSMT grant No. 2B06005.

Poster / Fungus. Tuesday, 10:30. **F-06**

***Metarhizium anisopliae* microsclerotia: Production and bioefficacy**

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Many plant pathogenic fungi produce sclerotial propagules or pigmented hyphal aggregates – termed microsclerotia (MS) – as overwintering structures. Hitherto, MS production by any of the entomopathogenic Ascomycetes has not been observed. Three strains of M*etarhizium anisopliae* (F52, TM109, and MA1200) produced these highly melanized hyphal aggregates in aerated, liquid cultures containing a basal salts medium supplemented with casamino acids and glucose to achieve a high carbon concentration (36 g/L) and carbon:nitrogen rations of >30:1. The highest concentrations of microsclerotia (2.7-2.9 x 10^8/L medium) were produced by strain F52. Air-dried, granular MS formulations readily germinated and sporulated on water agar plates, producting 5.3-11.4 x 10^8 conidia/g dried MS preparation. MS granules also grew out and sporulated abundantly in non-sterile clay and clay loam soil at moistures above -2.33 MPa. Soil incorporation of an MS granular formulation resulted in 100% mortality of third instar sugarbeet root maggot, *Tetanops myopaeformis* at rates as low as 1.9 g MS granules/1 Kg soil within one week. This rate approximated the concentration of granules when applied in furrow at the rate of 16.8 Kg/ha. The MS preparation retained viability for at least one year at 20-25° C. when stored dry and in vacuo.

Poster / Fungus. Tuesday, 10:30. **F-07**

Morphological characteristics and insect virulence bioassays of two high temperature adapted *M. anisopliae* strains

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One of the major impediments to the use of fungal pathogens for biological control is their relative low tolerance to abiotic stresses such as temperatures above their thermal limits (typically 32-34°C.). Using an automated continuous culture machine that actively selects for fast growing variants, *M. anisopliae* strain 2575 was adapted for growth at 37°C. Two thermotolerant clones, designated at *M.a.* 016 and *M.a.* 017, were isolated and their robust growth at 35-37°C confirmed *in vitro*. Morphological analysis of the isolates grown in liquid broth cultures revealed short stunted germ tubes for 016 at 37°C, whereas isolate 017 at 37°C appeared similar to WT (at 28°C). Isolate 017, when spread onto agar plates, began to penetrate the surface of the agar within 24 hr of growth, whereas the wild-type initially grew along the surface and began to penetrate the agar only after 48 hr of growth. Both isolates displayed decreased sporulation, however, with 016 producing ~25% and 017 less than 1% the number of spores per kg of solid substrate as the wild-type parent (WT). In topical bioassays at 28° C. using adult *Melanoplus sanguinipes*, 016 lost a significant amount of infectivity (as LD50) and virulence (as Median Survival Time), relative to WT; the original 017 could not be bioassayed because spore production was so poor. Passage of 017 through a grasshopper host restored sporulation to nearly WT levels. All three isolates failed to kill *M. sanguinipes* treated at the LD95-99 for each fungus and reared at 37° C, whereas the three killed their hosts within 5 days at 28° C.

Poster / Fungus. Tuesday, 10:30. **F-08**

Variability and identification of *Metarhizium* varieties and species based on heat tolerance, cold activity and molecular analysis

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Searches for effective new fungal biological-control agents for insects include not only obtaining new isolates, but also the identification of these to species and variety. This is particularly important for *Metarhizium* spp. targeted to locusts/grasshoppers and katydids (all Orthoptera), since *M. anisopliae* var. *anisopliae* (*M.an*) is pathogenic to many orders of insects, whereas *M. anisopliae* var. *acridum* (*M.ac*) is host specific, in that this variety attacks only Orthoptera. DNA-based techniques for identifying species and varieties are widely used, but these methods may be inconvenient or unavailable on some occasions. The current study suggests a simple, useful method based on tolerance to high and low temperatures to help identify some *M. anisopliae* isolates. Conidial suspensions of 37 *Metarhizium* isolates were exposed to wet-heat (45 ± 0.5°C) and plated on PDAY medium. After 8h exposure, the isolates could be divided into two groups. In group 1, all isolates of *M.an* and *Metarhizium* from the *flavoviridae* complex (*M.fl*) showed virtually zero relative germination (RG) of conidia, while *M.ac* presented high tolerance (ca. 70% to 100% RG). Furthermore, four *M.ac* isolates survived (ca. 40% to 70% RG) 24h exposure to the same temperature. The tolerance of isolates to low temperatures was also evaluated. All isolates exposed to 20°C and 15°C, for 2 and 7 days, respectively, showed high conidial germination. Isolates demonstrated high variability in RG when exposed to 10°C for 15 days with no respect for variety or species; however, when exposed to 5°C for 15 days, RGs for *M.an* and *M.ac* isolates were virtually zero, while the two *M.fl* were highly tolerant (100% RG). These *M.fl* isolates are probably *M. frigidum*. All of the isolates were analyzed by AFLP and rDNA sequencing to validate the identification of isolates. There was considerable genotypic variability in *M.an* isolates originating from the United States, but they all were clearly within the *M.an* group. In conclusion, heat and cold exposure can be used as tools to presumptively identify some important *Metarhizium* species and varieties, and to detect environmental conditions appropriate or limiting for each isolate.

Poster / Fungus. Tuesday, 10:30. **F-09 STU**

Comparison of new and commercial *Metarhizium* isolates based on multiple traits

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Metarhizium anisopliae, a common insect pathogen, is one of the most promising fungal species for biological control. Various isolates of *M. anisopliae* are known to demonstrate wide differences in tolerances to environmental factors, host specificity and virulence towards insects. The available commercialized fungal strains were selected primarily on their virulence and culturability. Both of these characteristics are important in the selection process, but expanding the selection criteria will be necessary to improve identification of isolates with presumptive high potential for development as an insect control agents. Since field trials are labor intensive, limited in scope, and expensive; the preliminary selection should be accomplished as much as possible in the laboratory. This study examines several characteristics as to their importance in identifying *M. anisopliae* isolates with high promise for pest biocontrol. Several new U.S. *M. anisopliae* var. *anisopliae* isolates (DWR 200, DWR 203, DWR 261, DWR 312, DWR 313, DWR 338, DWR 346, DWR 356) were compared with a commercialized isolate, F-52 (ARSEF 1095), and a commercialized *M. anisopliae* var. *acridum* (ARSEF 324) isolate. Traits considered included: virulence towards the orthopterous pest insect *Anabrus simplex* (Mormon cricket), culturability on rice, germination rates at several temperatures, growth rates, tolerances to wet-heat (45°C), and tolerances to UV-B radiation. The performance of the isolates varied between traits. Most of the var. *anisopliae* isolates killed *A. simplex* more quickly than the var. *acridum* isolate, but ARSEF 324 produced the highest number of conidia on rice, and tolerated heat and UV-B better than all the var. *anisopliae* isolates. While ARSEF 1095 has high virulence toward *A. simplex*, it was less tolerant to heat and UV and grew slower than many of the new isolates, indicating it may be less effective comparatively in the field than in the laboratory. Despite the slow insect mortality induced by ARSEF 324, this var. *acridum* isolate would be expected to be effective in the field due to its high tolerances to heat and UV-B irradiation. Of the var. *anisopliae* isolates, DWR 203 and DWR 346 would likely perform better then the commercialized product F-52. DWR 203, however, fails to produce sufficient conidia on inexpensive media for practical application. This study clearly demonstrates that, in addition to virulence and culturability, evaluating performance during and after exposure to environmental factors is essential for selecting fungal agents that will most effectively control target pests in the field.

Poster / Fungus. Tuesday, 10:30. **F-10**

Novel delivery of the fungus *Paecilomyces fumosoroseus* (Deuteromycotina: Hyphomycetes) for managing the Asian citrus psyllid (Psyllidae: Hemiptera): Laboratory investigation

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Efficacy of *Paecilomyces fumosoroseus* blastospores (Deuteromycotina: Hyphomycetes) on yellow tags *vs*. citrus leaves to manage Asian citrus psyllids, *Diaphorina citri* (Psyllidae: Hemiptera) were compared and horizontal transmission by psyllids among leaves assessed using a detached leaf bioassay. Psyllids were tested individually on either 4 leaf sections or 3 leaf sections and a treated yellow plastic tag. Treatments were citrus leaf sections or yellow tag of similar size (~100 mm^2) sprayed with *P. fumosoroseus*. Proportions of leaf sections were sprayed as follows: 25%, 50%, 75%, 100% compared to a yellow tag. Distilled water served as a control. A Fungal Development Index was used to determine infection rate once the insect had died and showed signs of mycosis. The yellow tag treatment was equally effective as the other leaf treatments in the rate of infection and spread infection more rapidly in psyllids compared to the 25% leaf treatment. Adults began to mycose at ~4-5 days post-release. As the inoculum increased for all leaf treatments the infection rate also increased. For all fungal treatments there was 100% horizontal transmission of *P. fumosoroseus* spores to all non-treated leaf sections. Inoculated yellow plastic tags may serve as an autodissemination technique for managing psyllid populations in citrus.

Poster / Fungus. Tuesday, 10:30. **F-11**

Potential of topical application, leaf residue and soil drench of fungus *Paecilomyces fumosoroseus* (Deuteromycotina: Hyphomycetes) for killing citrus weevil: Laboratory and greenhouse investigation

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The efficacy of different treatment applications with *P. formosoroseus* blastospore formulation (*Pfr* strain 3581) was assessed for potent to manage the citrus weevil, *Diaprepes abbreviatus*, DRW. *Pfr* was applied topically on the larvae and adults at a rate of 10^7 blastospores/mL and incubated in original rearing cups in the dark at 25˚C for 2 – 3 weeks. Another technique assessed feeding DRW detached citrus leaves previously sprayed with blastospores, after which DRW was incubated in Petri dish chambers at 25˚C for 2 – 3 weeks under 16 hr photophase. In the greenhouse, soil drench experiments were conducted to assess the effectiveness in killing the larvae. Each pot containing a citrus seedling drenched with 100 mL suspensions of *Pfr* at 10^7 blastospores/mL and then drenched with 400, 900 and 1,400 mL of water. Larval mortality due to the infection of *Pfr* was assessed after 2 weeks. *Pfr* may provide an added management tool against these difficult to control citrus pests.

Poster / Fungus. Tuesday, 10:30. **F-12**

Growth inhibition and revitalization of mycelia of *Paecilomyces tenuipes*, an entomopathogenic fungus

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To establish a stable growth inhibition and culture technique for *Paecilomyces tenuipes*, a study on the growth control and revitalization of its mycelia proliferated in pupa hosts was performed. All the mycelia that were developed in pupae and maintained at 4% moisture content survived. After they were freeze-dried or stored at temperatures of –70°C, –20°C, and 4°C for 14 days. Rehydration treatment for 3 hours resulted in the highest recovery rate, i.e., 94.3%~96.3%. Under the storage condition of 4% moisture content and 4°C temperature, the growth of the mycelia was stably inhibited until 135 days of storage. In the case of freeze drying (FD), the mycelia could be revived after 365 days. The optimum condition for synnemata formation was a temperature of 25°C and an illumination of 100~300 lux. Temperatures above 30°C or high-intensity illumination above 500 lux adversely affected synnemata formation. In a cross-match test under different light and temperature conditions, fruiting bodies with shapes similar to that of the teleomorph of *P. tenuipes* developed under certain light conditions; however, perithecial stromata were not formed. Thetemperature during culturing had no influence on the color and shape of the fruiting bodies.

Poster / Fungus. Tuesday, 10:30. **F-13**

Reassessment of vegetative compatibility groups (VCGs) in Japanese isolates of *Lecanicillium* spp.(*Verticillium lecanii*)

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Sixty-six isolates of *Lecanicillium* spp. (*Verticillium lecanii*) with diverse geographical origin and host were analyzed using restriction digestion of polymerase chain reaction amplified nuclear ribosomal DNA intergenic spacer (IGS) region. PCR-RFLPs using five enzymes divided into 21 IGS haplotypes. Ten haplotypes included more than one isolates. The remaining eleven haplotypes were unique haplotype that differed from other isolates. In Japanese isolates, isolates originated from whitefly expect for one isolate consisted of one haplotype, whereas the isolates derived from aphids showed various IGS haplotypes. Further, these isolates of *V. lecanii* were tested for vegetative compatibility by observing heterokaryon formation among complementary *nit* mutants. *nit* mutants were isolated from 65/66 strains examined. Twenty isolates were self-incompatible, and the resting 45 isolates were divided into 18 vegetative compatibility groups (VCG): eight containing only a single isolate each, and the remaining ten containing two to six isolates each. Members of isolates in each of all these VCGs except for one shared the same IGS haplotypes.

Poster / Fungus. Tuesday, 10:30. **F-14**

Ultraviolet light protection of *Lecanicillium attenuatum* with titanium dioxide

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The exposure of conidia to sunlight, especially to the UV component, can cause serious reduction of viability; and this is one of the major obstacles to use of entomopathogenic fungi for microbial control. Formulation of conidia can be important to protection of conidia from UV in field application of mycoinssecticide. In the present study, susceptibility of *Lecanicillium attenuatum* CS625 conidia to UV-B irradiation with or without formulation compounds was investigated. Conidia were spread on Petri plates of agar medium, the open plates were covered with cellulose diacetate film (which blocked radiation below 290nm), and exposed to UV-B irradiation for 1, 2, or 4 hours. Total doses were 2.9, 5.8, and 11.8kJm-2, respectively. Conidia of *L. attenuatum* were highly susceptible to UV-B irradiation. About 80% of unformulated (unprotected) conidia were killed after 1-hour irradiation, and all spores were killed after 2 hours of UV-B exposure(PROC GLM: F=89.98; df=3, 8; Pr>F=<0.0001). Several compounds were mixed with conidia and tested as UV protectant for *L. attenuatum:* three types (water-dispersible photostabilized, water-indispersible photostabilized, and water-soluble photounstabilized) of titanium dioxide, charcoal, yeast extract, and beef extract . The three forms of titanium dioxide maintained the viability of *L. attenuatum* conidia, but no protection was provided by charcoal, yeast extract, and beef extract. In UV-B irradiation treatments for 1, 2, and 4 hours, 0.5% water- soluble photo-unstabilized titanium dioxide was the most protective material. After 2 and 4 hours of UV-B irradiation, the culturability of *L. attenuatum* formulated with water-soluble photo-unstabilized titanium dioxide was 74% and 28% compared with 0% for unformulated spores. These results indicate thatsome formulation compound, particularly water-soluble photo-unstabilized titanium dioxide can protect conidia from UV-B irradiation; and their presence may crucial to the success of these fungi on solar-exposed plant surfaces.

Poster / Fungus. Tuesday, 10:30. **F-15 STU**

***Lecanicillium* spp. (= *Verticillium lecanii*) penetrate into *Trialeurodes vaporariorum* egg**

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In our previous study of bioassays for different developmental stage of *Trialeurodes vaporariorum* by *Lecanicillium* spp., we have found severely- deformed eggs that covered with fungal hyphae and dead hatching larvae on the eggshell. Although several earlier study on infectivity of *Lecanicillium* spp. to egg phase have reported that eggs were not invaded by *Lecanicillium* spp., our observation of this phenomena have indicated the possibility of fungal activity influenced to eggs and/or hatching. Then, we surveyed the degree of penetration of *Lecanicillium* spp. to eggs and hatchability at 15, 20 and 25°C. Consequently, approximately 10% of eggs were invaded at all temperature regimes and during this experiment the rate of penetration was steady at 10%. One interpretation for this stability of penetration rate is presence of the phase being able to invade. On the other hand, hatchability of *Lecanicillium* sp. treated eggs indicated approximately 10% lower than that of Control at 20 and 25°C. It can be presumed that ovicidal activity or hatch inhibition by fungal treatment are held responsible for the lower hatchability. We will discuss the penetration of *Lecanicillium* spp. to eggs from the relation of the fungal strains and timing for application.

Poster / Fungus. Tuesday, 10:30. **F-16 STU**

Preventive application to control cotton aphids and greenhouse whiteflies by *Verticillium lecanii* (=*Lecanicillium* spp.)

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In the previous study, protoplast fusion has been performed with 3 strains of the entomopathogenic fungus *V. lecanii* (Aiuchi et al.,2008). Some hybrid strains showed high pathogenicity against cotton aphids and greenhouse whiteflies, and high viability on leaf surface under low humidity condition. In the present study, we investigate the efficacy of preventive application and direct application against cotton aphid and greenhouse whitefly. Bioassay for cotton aphid; on weekly sprays of 2aF43 showed no change to aphid population and Vertalec has almost eradicated aphids. Preventive application of 2aF43 and Vertalec decreased the aphid population slightly. Bioassay for greenhouse whitefly; preventive application showed higher mortality of hatched first instar nymph than direct application to first instar nymphs. In addition whiteflies laid significantly fewer eggs on the leaf with fungal treatment than those of control. It is suggested that greenhouse whitefly might avoid fungal treated leaf to oviposit. More over 2aF43 treatment induced significantly lowest hatchability of them. It can be presumed that *V.lecani* has ovicidal effect or hatching inhibition. Hence, preventive application of *V.lecanii* especially affects in controlling early immature stage of greenhouse whitefly and the egg phase might be a new target stage.

Poster / Fungus. Tuesday, 10:30. **F-17 STU**

Sporulation of *Verticillium lecanii* (=*Lecanicillium* spp.) on cotton aphid cadaver in different humidity conditions

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Verticillium lecanii(*Lecanicillium* spp.) requires high relative humidity(RH) to control the pests and usually the transmissions occur by contact. In this study, the experiments were conducted to estimate the sporulation on cotton aphid cadaver in different RH conditions. Vertalec, Mycotal, B-2 and two hybrid strains (2aF4, 43) were used. The aphids killed by ethyl acetate were immersed to conidial suspension(x 10^7 conidia/ml) and put into assay cages with salt or glycerol solution in a bottom to control RH. The amounts of conidia were detected by dilution plating method. 100% RH exhibited significantly higher sporulation(1.53-3.38 x 10^5 cfu/cadaver) and at 98% RH also exhibited abundant sporulation(1.04-8.09 x 10^4 cfu/cadaver) except B-2 strain, while under lower RH, existence on cadaver were confirmed but hyphal growth couldn't be observed from cadaver. Among tested strains, the modes of conidiation were clearly differed. Vertalec and 2aF43 produced sparse hypha with many spore heads, while Mycotal and B-2, 2aF4 produced more fluffy hypha with no spore heads. Vertalec and 2aF43 produced many conidia on cadaver and the mode of conidiation might be useful to spread the infection.

Poster / Fungus. Tuesday, 10:30. **F-18**

Beneficial associations between *Pandora neoaphidis* and noncrop plants inhabiting lettuce field margins

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Field margins composed by noncrop plants are important in annual cropping systems as lettuce, where the rate of establishment of aphid pests and entomopathogenic fungi are limited in space and time. To identify the noncrop plants that act as reservoirs of entomopathogenic fungi adjacent to a lettuce crop, a survey on different plant species was made to search for aphids infected by entomopathogenic fungi. Samplings on noncrop plants were carried out in La Poveda (Arganda del Rey, Madrid) during lettuce growing season in Spring 2007 and 2008. Field margins adjacent to lettuce were composed by herbaceous plants belonging to eight families in which grasses were predominant. *Acyrtosiphon lactucae*, *Aulacorthum sp.*, *Metopolophium festucae* and *Ropalosiphum padi* killed by *Pandora neoaphidis* were found on *Malva sylvestris*, *Sylibum marianum*, *Myosotis arvensis* and *Hordeum murinun*. Aphid species unable to colonize lettuce were found infected by *P. neoaphidis* on noncrop plants in mid-March, two weeks earlier than infections were detected on lettuce aphids. Initial infections by *P. neoaphidis* on aphid species colonizing lettuce were recorded on plants located in the first rows of the crop, close to this field margin, suggesting the importance of field margins for the beginning of epizootics of *P. neoaphidis* on lettuce aphid pests.

Poster / Fungus. Tuesday, 10:30. **F-19**

Spatial distribution of aphids infected by *Pandora neoaphidis* and aphidophagous syrphids in lettuce crops

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The entomopathogenic fungus *Pandora neoaphidis* and aphidophagous syrphids are the main natural enemies reducing aphid populations on lettuce crops. The aim of this work was to study the spatial and temporal patterns of these two natural enemies within lettuce crops. Experiments were carried out on a lettuce crop located in La Poveda (Madrid) during Spring 2007 lettuce growing season (March to May). One hundred and twenty lettuce plants regularly distributed within the crop were sampled once a week. Aphid population, the number of cadavers infected by *P. neoaphidis* and the number of aphidophagous syrphids were recorded on each plant sampled. Data of the spatial distribution of natural enemies were analized by SADIE methodology. The occurrence of aphids attacked by *P. neoaphidis* and colonization of syrphid larvae started from two different edges of the crop. *P. neoaphids* infections on aphids began in mid-March; however syrphids colonized the crop four weeks later. Lettuce plants supporting both syrphid larva and *P. neoaphidis*-infected *Nasonovia ribisnigri* and *Macrosiphum euphorbiae* were observed from mid-April to end of May (harvest). Significant spatial association between *P. neoaphidis* and syrphids occurred throughout the whole month of May. The highest density of both natural enemies occurred in mid-May. Results showed that *P. neoaphidis* and syrphids may coexist together on lettuce plants following similar spatial patterns.

Poster / Fungus. Tuesday, 10:30. **F-20**

Transmission of *Pandora neoaphidis* in the presence of co-occurring arthropods

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The transmission of the entomopathogenic fungus *Pandora neoaphidis* to aphids is enhanced in the presence of arthropod guild members such as coccinellids and parasitoids. Here we assessed whether insects that co-occur with the fungus, but are not natural enemies of the aphids in the habitat, also have an effect on the transmission of *P. neoaphidis*. The presence of foraging Peacock butterfly (*Inachis io*) caterpillars significantly increased the transmission of *P. neoaphidis* to nettle aphids, *Microlophium carnosum*, on excised nettle leaves, despite the caterpillar indirectly reducing the number of available aphids by >30%. The effect of caterpillars on the transmission of *P. neoaphidis* is, therefore, likely to be dependent on the extent of herbivory. In cage arenas the transmission of *P. neoaphidis* to the nettle aphid was enhanced in the presence of the non-enemy parasitoid *Aphidius rhopalosiphi* to a level similar to that in the presence of the enemy parasitoid *A. microlophii*. It is likely that the increase in transmission in the presence of *I. io* and *A. rhopalosiphi* is due to disturbance and subsequent movement of the aphid, resulting in contact with conidia deposited on the leaf surface. The presence and impact of co-occurring arthropods should be taken into consideration when assessing the transmission of fungal entomopathogens.

Poster / Fungus. Tuesday, 10:30. **F-21**

Molecular characterization of entomopathogenic fungi using microsatellite markers

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Identification of entomopathogenic fungi isolated from their arthropod hosts or other sources can be cumbersome when certain morphological features are not clear or time consuming when the organism needs to reach a certain growth stage for proper identification. Molecular tools can be handy and offer a faster solution under such circumstances. Microsatellite markers were used to examine molecular characterization of two isolates each of *Beauveria bassiana* and *Metarhizium anisopliae*, as well as four isolates of *Hirsutella homalodiscae*. These isolates were recovered from insect hosts or soil from California, Texas, Mississippi and Florida. Microsatellite markers Ba03, Ba05, Ba06, Ba08, and Ba13 successfully separated the fungal species and their isolates. These results demonstrate the effectiveness of microsatellites and the high transferability of these markers across other fungi based on polymerase chain reaction product-amplification using markers developed specifically from Genomic DNA of *B. bassiana* by the USDA-ARS Insect Biocontrol Laboratory Beltsville, MD. In addition, these results suggest conservation of specific repeat motifs and/or genomic region among fungal species and isolates.

Poster / Fungus. Tuesday, 10:30. **F-22**

Isolation and characterization of a photolyase gene from the entomopathogenic fungi *Beauveria bassiana.*

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UV radiation causes the formation of cyclobutane pyrimidine dimers (CPDs) in DNA. In fungi, CPDs are responsible for mutations, inhibition of spore germination and growth retardation, which can affect field performance in biological control applications. To improve UV resistance in *Beauveria bassiana*, the gene that encodes a CPD photolyase, an enzyme that catalyzes the repair of CPDs, was isolated and characterized. Primers that align to homologous protein regions were designed, and together with genome walking, the complete sequence of the *phr1* gene was amplified from genomic DNA of the strain Bb9205. The 1933 bp transcript of the gene consists of two exons and one intron of 52 pb. The sequence encodes a putative protein of 626 amino acids, with a 59-70% similarity to CPD class I photolyases present in other fungi. The 3D structural model of the protein resembles the DNA photolyase from *Escherichia coli*, conserving the FAD (Flavin Adenin Dinucleotide) and the methyl 5, 10 methenyl-6 hydropholic acid binding sites, characteristic of this protein family. A full length cDNA amplified from mRNA extracted from mycelium exposed to UV-B/UV-A and visible light was engineered into the fungal transformation vector pBAR.GPE1 for over-expression in the same *B. bassiana* strain. Co-financed by the Colombian Ministry of Agriculture and Rural development.

Poster / Fungus. Tuesday, 10:30. **F-23**

Identification of transcripts with increased expression during conidiogenesis of the entomopathogenic fungus *Metarhizium anisopliae*

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Conidia are responsible for the reproduction, dispersal and environmental persistence of different fungal species of medical, industrial and agricultural interest. In pathogenic species such as *M. anisopliae*, conidia also are the structures responsible for host infection. The identification of new genes expressed during conidiogenesis will provide important information for the understanding of the biology of this fungal structure. We used suppressive subtractive hybridization (SSH) to isolate transcripts with increased expression during conidiogenesis of the ARSEF 324 strain of *Metarhizium anisopliae* var. *acridum*. A total of 212 expressed sequences tags (ESTs) were obtained. The sequencing and clustering of ESTs, which correspond to the genes with increased expression during conidiogenesis, permitted the identification of 54 genes of the fungus, only one of which (the G3PDH gene), had been previously described in this species. The increased expression of three of these genes (a hydrophobin gene, the gene of a protein associated with senescence and an unknown sequence that has an active site present in thiolases) during conidiogenesis was confirmed by quantitative real time RT-PCR. Functional characterization showed that most of the genes have unknown functions or code for hypothetical proteins (48 and 9 %, respectively). The description of 53 new genes expands the number of genes known in *M. anisopliae*.

Poster / Fungus. Tuesday, 10:30. **F-24**

Avoidance of entomopathogenic fungi by insect predators

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Insects can detect cues related to the risk of attack by their natural enemies; including entomopathogenic fungi. Behavioural mechanisms that enable insects to avoid infection by fungal pathogens would be advantageous adaptations. We conducted experiments to assess the potential of common insect predators to detect and avoid their fungal natural enemy *Beauveria bassiana*. The predatory bug *Anthocoris nemorum* avoided nettle leaves treated with *B. bassiana*, and females laid fewer eggs on leaf halves contaminated with the pathogen. Adult seven spot ladybirds, *Coccinella septempunctata*, overwinter in the litter layer often in groups. Adult *C. septempunctata* modified their overwintering behaviour in relation to the presence of *B. bassiana* conidia in soil and sporulating conspecifics by moving away from sources of infection. Furthermore, active (non-overwintering) adult *C. septempunctata* detected and avoided *B. bassiana* conidia on different substrates, including leaves and soil. Our studies show that insect predators have evolved mechanisms to detect and avoid pathogens that they are susceptible to. Fungal pathogens may be significant mortality factors among populations of insect predators, especially long-lived species that must diapause before reproduction. Likewise, actively foraging species are more likely to come in contact with pathogens than predators that sit and wait for prey.

Poster / Fungus. Tuesday, 10:30. **F-25**

Isolation of entomopathogenic fungi from soil collected from western United States

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The Mormon cricket (MC), *Anabrus simplex*, is an important agriculture pest in western United States. Our current project on MC biology and diseases includes a search for new fungal isolates with greater potential to control MC than currently available commercial isolates. Cadavers of insects infected in nature are difficult to find because of the cannibalistic habits of MC and the presence of numerous scavengers. Similarly, holding field-collected MC in the laboratory until death yields few insect-pathogenic fungi from surface sterilized cadavers. Accordingly, since fungus-killed MC/Grasshopper (GH) should deposit conidia on or in nearby soil; our survey has centered on isolating fungi from soil collected in MC/GH habitats. During 2007, 732 soil samples were collected in North Dakota (306 samples), Arizona (197), South Dakota (147), Utah (62), Montana (12), Idaho (7), and Virginia (2). Most of the soil samples were collected by the MC/GH Population Survey Team of each state. Small sub-samples were weighed, suspended in sterile double distilled water, and 50 ml aliquots inoculated onto a medium selective for entomopathogenic fungi [Potato Dextrose Agar plus Yeast Extract (PDAY) with 0.002% or 0.004% Dodine (n-dodecylguanidine acetate)] in Petri plates. Seventeen new *Metarhizium anisopliae* isolates (13 North Dakota, 2 Arizona, 1 South Dakota, and 1 Virginia), 63 *Beauveria bassiana* (33 North Dakota, 11 South Dakota, 11 Arizona, 8 Utah), and 12 *Trichoderma* sp. (3 South Dakota, 3 Arizona, 2 North Dakota, 2 Montana, 1 Utah,

1 Idaho) have been isolated. The identifications were based on morphology, and the isolates are stored at low temperature. Further studies will identify the isolates using DNA-based techniques, isolates with high virulence to MC by laboratory assays, will be detected, and the isolates will be tested for high tolerance to environmental conditions, such as heat and ultraviolet light, routinely encountered in MC habitats. The project has been expanded significantly this year (2008), based partially on an encouraging outdoor cage trial in 2007 of a *Metarhizium* isolate obtained from Arizona soil in 2005 using the selective-medium approach. The isolation technique is under revision to further enhance recovery of an Orthoptera-specific fungal variety, *M. anisopliae* var. *acridum*.

Poster / Fungus. Tuesday, 10:30. **F-26**

Survey for entomopathogenic fungi from *Rhynchophorous ferrugineus* (Oliv.) (Coleoptera, Curculionidae)

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A survey for etomopathogenic fungi in the red palm weevil (*Rhynchophorus ferrugineus* (Olivier)) (Coleoptera: Curculionidae), infested *Phoenix canariensis* Hort. was conducted in Sicily (Italy), where this beetle was recently introduced. The RPW is one of the most important pests of the palm trees, its larvae feed on plant tissues causing frequently the death of the tree. Because of the concealed nature of the larvae, effective methods for the management of the red and other palm weevils have been difficult to develop. Thus there is now a strong emphasis on the development of integrated pest management (IPM) based on pheromone traps and biological control rather than insecticides. Pupae, larvae and adults were collected from infested palms in spring 2008. The natural incidence of the entomopathogenic fungi was recorded on RPW. The entomopathogenic fungi were found mainly in the pupae. Parasitism was about 30% of the collected pupae. Symptoms of fungal infection included rapid pupae tissue atrophy and failure of adults to emerge, death. Dissections of infected pupae revealed dense hyphal growth inside pupae, thus suggesting fungal penetration and pathogenicity. Fungus culture was collected from the infested insects, cultured on nutrient agar and characterized both by specific molecular markers (e.g. microsatellites and ITS) and microscopical analysis (SEM, CLSM).

Poster / Fungus. Tuesday, 10:30. **F-27 STU**

Induction of defense-related genes in banana (*Musa* spp.) by endophytic *Fusarium oxysporum*

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The plant-parasitic nematode *Radopholus similis* and the banana weevil *Cosmopolites sordidus* are major pests of banana (*Musa* spp.) in East Africa. Some naturally occurring endophytic Fusarium oxysporum strains, when reintroduced into sterile tissue-cultured banana plants, have the ability to reduce pest populations. Studies on the mode of action of these two endophytic strains, particularly against the nematode, have implicated the induction of systemic resistance in banana plants. To identify molecules induced upon endophyte colonization and pest infestation, gene expression studies were carried out in banana cultivars susceptible and tolerant to *R. similis* following earlier inoculation of plants with strains V5w2 and Emb2.4o. Results indicated up-regulation of genes involved in cell wall strengthening, signal transduction and induced systemic resistance. In a related study, the expression of eight putative banana defense-related genes was investigated in susceptible and tolerant banana cultivars following inoculation of strain V5w2 with *R. similis* challenge. The expressions of PR1 and catalase genes were up-regulated upon *R. similis* challenge of plants of the tolerant cultivar previously inoculated with the endophyte. The two studies provide the first report of molecular elucidation of fungal endophyte-induced resistance in banana.

Poster / Fungus. Tuesday, 10:30. **F-28**

Observations of fungal disease in the giant willow aphid (*Tuberolachnus salignus*)

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Willow (*Salix* spp) grown as short rotation coppice (SRC) is one of the main biomass crops in the UK. Willows are the host plant of the giant willow aphid, *Tuberolachnus salignus*, which often occurs at high density. The predicted expansion of willow plantations, and the possibility that springs and summers may become drier, have increased concern that these aphids may become a serious pest problem on SRC. Large numbers of the giant willow aphid are often killed in autumn by an entomopathogenic fungus. There is uncertainty about the identity of this fungus, which is likely to be from the genus *Neozygites*, but is possibly a hitherto undescribed species. The development of fungal infection in *T. salignus* populations occurs rapidly, but the mechanism of transmission is not understood as only resting spores have been observed and not infectious conidia. Field observations and laboratory experiments with resting spores are underway to elucidate the fungus' life cycle and virulence.

WEDNESDAY - 6 August

SYMPOSIUM (Bacteria Division) Wednesday, 8:00–10:00

Entomopathogenic Bacteria Other than *Bacillus*

Symposium. Wednesday, 8:00. **90**

Drosophila host defence against *Pseudomonas entomophila*

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The Drosophila innate immune system is remarkably efficient at controlling pathogen infection and has proven to be a valuable model for the investigation of host-pathogen interactions. Our understanding of the Drosophila response to bacterial pathogens has focused on direct injection of bacteria into the body cavity of the insect, thereby by-passing the more natural infection route of oral ingestion. Additionally, studies have been hampered by the lack of true Drosophila pathogens. We previously identified a novel bacterial species, *Pseudomonas entomophila,* that leads to the death of both Drosophila larvae and adults by oral infection. However the relationship between *P. entomophila* virulence and Drosophila death is yet to be determined. Upon ingestion of *P. entomophila*, Drosophila triggers a strong local and systemic immune response leading to a massive expression of antimicrobial peptide (AMP) genes. Despite this host specific answer to a gram negative bacteria infection *P. entomophila* is able to persist within the insect gut; this persistence is associated with dramatic cytopathologies at the level of the epithelial intestinal cells. Genome comparison to other *Pseudomonas* pathogens has identified several features that may contribute to *P. entomophila* entomopathogenic properties, including insecticidal toxins, proteases, lipases, hydrogen cyanide, lipopeptides and other secondary metabolites. Most of the identified potential virulence factors appear to be regulated by the two-component system GacS/GacA as has been demonstrated for the metalloprotease AprA that contributes to *P. entomophila* resistance to *Drosophila* AMPs. *P. entomophila* virulence is believed to be a multi-factorial mechanism in which the ability of the bacteria to counteract and/or subvert the host defense might have a central role. Thus the *P. entomophila*/*Drosophila* interaction represents a tractable model to address major questions at the level of both bacteria virulence factors and invertebrate immunity.

Symposium. Wednesday, 8:30. **91**

Virulence determinants of *Yersinia entomophaga* MH96: a genomic perspective.

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A unique insecticidal bacterium designated *Yersinia entomophaga* MH96 isolated from the New Zealand grass grub *Costelytra zealandica.* Host range testing showed that the bacterium has broad insecticidal activity, killing a number of Lepidoptera and Coleoptera species within 3 to 5 days post infection. An overview of this unique bacterial pathogen will be described from host range to mode of action. Preliminary data from the partially completed genome sequence of *Y. entomophaga* will be described; including an evolutionary perspective on the relationship of *Y. entomophaga* to *Yersinia pestis, Yersinia pseudotuberculosis* and *Photorhabdus luminescens*. Facets of the genome's predicted virulence systems; 1) genomic islands including pathogenicity islands, 2) tc (toxin complex) loci, 3) type III secretion system, 4) *rhs* genes that may serve as recombination hot spot or may encode toxins, 5) Rtx toxins, 6) hemolysin- or adhesin-related proteins and 7) insect defence systems, will be discussed. In addition, potential mobile elements including bacteriophage elements and unique parts of the genome will be described. Genomic analysis of *Y. entomophaga* will allow us to target particular virulence related genes and assess their role in an insect model system for fundamental and applied research, including studies of host-bacterial interactions.

Symposium. Wednesday, 9:00. **92**

Insecticidal toxins from *Photorhabdus*: Comparative genomics and Rapid Virulence Annotation (RVA)

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The genomes of bacteria in the genus *Photorhabdus* encode numerous novel toxins including members of the Toxin complex (Tc) family, Makes Caterpillars Floppy (Mcf) toxins, PirAB toxins and many others. Following the recent completion of two complete genome sequences, one from *P. luminescens* and one from *P. asymbiotica*, we will describe the comparative genomics of toxin encoding genes in these two very different pathogens. We will also discuss what we know about the mode of action of these different toxin classes. Finally, we will describe novel massively parallel screens to perform functional annotation of novel toxin genes in bacteria. We have termed this approach Rapid Virulence Annotation or RVA and its applications to bacteria other than *Photorhabdus* will be discussed.

Symposium. Wednesday, 9:30. **93**

Pathogenesis of *Serratia entomophila* (Enterobacteriaceae) towards the New Zealand grass grub *Costelytra zealandica*.

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Serratia spp. (Enterobacteriacae) are commonly isolated from grassland soils. In New Zealand, isolates of *S. proteamaculans* and *S. entomophila* contain a specific plasmid (pADAP) encoding genes imparting pathogenicity to the grass grub, *Costelytra zealandica* (Coleoptera: Scarabaeidae). Ingestion of bacteria and organic matter into the high pH gut of the larva induces production Sep proteins, which are related to the Tc complex found among the Enterobacteriaceae. An anti-feeding prophage is also released. Disease follows, with a rapid cessation of feeding, disruption of the protease synthesis pathway and clearance of the midgut. While disease symptoms are concentrated within the midgut, there is no evidence of bacterial colonisation or damage to the midgut cells. The host responds to the release of bacterial proteins by the expulsion of the gut contents to the hindgut and the release of frass pellets. Once

the first flush of disease is completed, the infected larva remains in an active but non-feeding state for some weeks until fat reserves are consumed, and internal tissues weaken, leading to bacterial invasion of the haemocoel causing septicaemia and death of the host. Release of bacteria into the soil ensures a recycling of disease with pathogenic strains commonly found among old, declining populations of grass grub. While belonging to the Tc toxin family, the New Zealand *Serratia* Septoxins only cause disease in *C. zealandica*. A strain of *S. entomophila* is successfully being used as a commercial control for the grass grub in pastures and horticultural crops in New Zealand.

SYMPOSIUM (Microsoridia Division) Wednesday, 8:00–10:00

Microsporidia of Aquatic Arthropods

Symposium. Wednesday, 8:00. **94**

Microsporidian parasite of caddis flies (Trichoptera) with comment to phylogeny and classification of Microsporidia in general

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Twelve microsporidian species infecting caddis fly larvae including genera Episeptum, Paraepiseptum (formerly Pyrotheca, Cougourdella), Zelenkaia (provisionally designated genus), Issia and Amblyospora (formerly Thelohania) were characterised. All studied species belonging to genera Episeptum, Paraepiseptum and Zelenkaia are host-species specific parasites infecting fat body and oenocytes of their hosts. Their spores are not infective for the original host, their life cycle involves an intermediate host and (or) transovarial transmission. Data obtained by rDNA sequencing showed that microsporidia from Trichoptera form several separate groups within a large clade uniting microsporidia from crustacea (first of all Copepoda and Cladocera) and insects with aquatic larval stages (Diptera - Culicidae, Simuliidae). It occurs that the presently known microsporidia from Trichoptera have no common and direct ancestor in their evolution. It is hypothetized that microsporidia invaded individual groups independently, but always from ancestors parasitizing crustacea. The presence and mosaic distribution of crustacean microsporidia across all clades of phylogeny trees suggests that the model of their radiation from crustacean hosts was probably repeated in other groups of hosts who acquired microsporidia from crustacea living with them in the same environment.The life cycles of microsporidia switching insects and crustacea as their hosts (e.g. mosquito Amblyospora spp.) are probably relics of the original radiation event. Phylogeny data suggests that microsporidia from Trichoptera could be of similar type.

Symposium. Wednesday, 8:20. **95**

Evolutionary interactions between microsporidia and their hosts: Lessons from an ancient lake

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Parasites are an indispensable part of any ecosystem and have strong influences on the ecology and evolution of their hosts. It has been testified that there are strong positive correlation between host species richness and parasite diversity and parasites are suggested to play a significant role in host diversification. However there have been no specific studies of parasite diversification within a host species radiation. Lake Baikal, in Russia, is the largest and oldest continent freshwater lake (c27MY) in the world. This isolated ecosystem harbours at least 260 unique endemic amphipod species, which have diverged within the lake since it originated and often cited as a classic case of adaptive radiation. The age, diversity and isolation of Baikalian amphipods provide a perfect system to study the host-parasite evolutionary interactions. We have therefore conducted a specific survey to measure the diversity and distribution of microsporidian parasites within Baikalian amphipods collecting 31 amphipod species, selected to represent mass species with the widest range of adaptations. PCR based screening using universal microsporidian SSU rDNA primers demonstrated that all species were infected by microsporidia. Sequencing of PCR products revealed high diversity of parasites with over 100 novel microsporidian sequences. Phylogenetic reconstruction shows these parasites are highly diverse with multiple origins. Two clades of microsporidia are overrepresented in Lake Baikal and within one of these there is support for the presence of endemic parasite species flocks. Microsporidia are a diverse phylum of intracellular parasites. They are not only important pathogens of amphipods, but can also affect host population dynamics by host sex ratio distortion and can influence both community structure and the likelihood of biological invasions. Analysis of the trophic interactions which occur between these parasites in Baikalian amphipods will allow us to assess the importance of these parasites to community structure and biodiversity maintenance.

Symposium. Wednesday, 8:40. **96**

Microsporidia in freshwater Amphipods: an overview and an example

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Microsporidia from freshwater Amphipods were only recently the focus of intensive research. Based on a literature review, an overview will be first presented, including data on molecular systematic, transmission (vertical vs. horizontal), fitness effects for the host (feminization…), host ranges and geographical distribution. Parasitism as a component of the success and dynamic of invasions, as well as the potential impact of alien parasites on the local fauna are regarded as important factors. Therefore, this paper will also present ongoing research on a newly characterised microsporidia, *Microsporidium sp. D*, infecting the invasive amphipod *Dikerogammarus villosus*. Originating from the Ponto-Caspian area, *D. villosus* invaded almost all large rivers of Europe in less than 30 years. PCR-RFLP typing showed that *M. sp. D* followed its host almost all along its invasion route. In an attempt to test if *M. sp. D* is a way to control the invader or/and a risk for the local fauna, results about transmission (vertical vs. horizontal) and potential impact on host fitness as well as prevalence and pathogenicity to the local fauna will be presented for two invaded areas contrasting by their invasion history and local fauna: Burgundy (France) and Eastern Poland.

Symposium. Wednesday, 9:00. **97**

Coevolutionary dynamics of host-parasite interactions in natural *Daphnia* populations

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Parasites have a negative effect on the reproduction and survival of individual *Daphnia*, which is translated in population level effects.

The *Daphnia* mostly do not evolve as fast as their parasites, however, adaptive genetic changes occur upon infection. In their 'arms race' against the fast evolving parasites, there will be selection in the *Daphnia* against defence mechanisms, other than those that are abundant in the momentary interaction, as *Daphnia* parasites adapt to specific abundant host genotypes. The antagonistic interactions between the waterflea *Daphnia* and its parasites are a key structuring force in natural populations, driving their coevolution. Direct empirical demonstration of long-term host-parasite coevolution, in particular Red Queen dynamics, is difficult. Here we capitalize on the fact that dormant stages of both parasites and hosts of our model system, the waterflea *Daphnia* and its micro-parasites, are conserved in lake sediments and thus provide an archive of past evolutionary dynamics. This allowed us to reconstruct host – parasite coevolution in a natural setting. We document evidence for fast temporal adaptation of the parasite, which supports the idea of ongoing coevolution between the host and the parasite.

Symposium. Wednesday, 9:20. **98**

Epizootiological studies of *Amblyospora camposi* (Microsporidia: Amblyosporidae) in *Culex renatoi* (Diptera:Culicidae) and *Paracyclops fimbriatus fimbriatus* (Copepoda:Cyclopidae) in a bromeliad habitat

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The epizootiology of *Amblyospora camposi* was studied in a natural population of *Culex renatoi*, a bromeliad-inhabiting mosquito, and its intermediate host, *Paracyclops fimbriatus fimbriatus*, over a 2-year period. Twenty *Eryngium cabrerae* plants were sampled monthly and the prevalence of *A. camposi* in *P. f. fimbriatus* and *Cx. renatoi* populations was determined. The monthly prevalence rates of meiospore infections in *Cx. renatoi* larvae never exceeded 5,5% and was detected in 50% of the monthly samples. Meiospores were available in plants over the course of the study at a mean concentration of 2×10^{4} meiospores/ml. Within each plant the parasite was maintained by horizontal transmission. *P. f. fimbriatus* with vegetative stages and mature spores were found regularly in bromeliads suggesting efficient meiospore infectivity to field copepod populations. The mean concentration of spores from copepods found in plants was 8×10^{2} spores/ml. Infections in copepods were detected in 54% of the monthly samples with a prevalence rate ranging from 0,55 to 17,4% and an overall average of 5,1%. Vegetative stages in fourth instar mosquito larvae (probably derived from the horizontal pathway via spores formed in copepods) were detected in 12.5% of the monthly samples with an overall prevalence rate of 1,1%. Infections in female and male adults were detected in 20,8% of the monthly samples with an overall average of 4,1% and 6,8% respectively. The host – parasite relationship of *A. camposi* could be yet another example of how a microsporidium has adapted to the ecological parameters of its hosts and the specialized habitat where they are found in nature to ensure long term survival.

Symposium. Wednesday, 9:40. **99**

Intranuclear microsporidians in crustaceans: The genus Enterospora

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To date, only one genera (*Nucleospora*) within one family (Enterocytozoonidae) of microsporidians contain species that are parasitic within the nuclei of their host cells. All described intranuclear species of the genus *Nucleospora* exist in fish. We have recently discovered the first intranuclear microsporidian parasite within an invertebrate, the European edible crab (*Cancer pagurus*). Whilst infected crabs displayed no obvious external symptoms, histologically, infected hepatopancreatic tubules were characterised by the presence of varying numbers of hypertrophic and eosinophilic nuclei within epithelial cells. Parasite stages appeared as eosinophilic granular accumulations causing margination of host chromatin. In advanced cases, degeneration of the tubule epithelia occurred, with parasites and sloughed epithelial cells appearing in tubule lumens. Ultrastructurally, all life stages of the parasite were observed within host nuclei. Uninucleate meronts were not detected though bi-nucleate stages were observed. Multinucleate plasmodia (sporogonal plasmodia) contained up to 22 nuclei in section. Significantly, late stage plasmodia contained multiple copies of apparatus resembling the polar filament and anchoring disk that appeared to associate with individual plasmodial nuclei. As such, aggregation and early assembly of sporoblast components took place *within* the intact sporogonial plasmodium, a unique feature of the family Enterocytozoonidae. Liberation of sporoblasts from plasmodia or the presence of liberated sporoblasts was not observed in this study. However, maturing and mature spores were observed in direct contact with the host nucleoplasm. In many cases, large numbers of spores were observed within a given section of host nucleus. Spores measured 1.3 ± 0.02 x 0.7 ± 0.01 mm. By considering the shared features of this parasite with microsporidians of the family Enterocytozoonidae and the unique presence of this parasite within the nucleoplasm of hepatopancreatocytes from a decapod crustacean, it was proposed that this parasite is the type species of a new genus of microsporidian (genus *Enterospora*). Further work is now required to provide molecular taxonomic data for comparison of *Enterospora* to the two other genera, *Enterocytozoon* and *Nucleospora* that reside within the Enterocytozoonidae and with other hepatopancreatic microsporidians of crustacean hosts.

CONTRIBUTED PAPERS Wednesday, 8:00-10:00

FUNGI 2

Contributed paper. Wednesday, 8:00. **100**

Genetic analysis of conidiation mutants in *Metarhizium anisopliae* derived by *Agrobacterium*-mediated mutagenesis

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We are interested in understanding genes involved in fungal conidiation. Entomopathogenic fungal conidia are both the infectious propagules responsible for initiating infection as well as the dispersal unit for spreading disease. We screened a library of Agrobacterium-mediated mutants of Metarhizium anisopliae for aberrant colony morphology and conidiation phenotypes. Successful insertion of the T-DNA fragment was screened using a positive

selectable marker (phosphinothricin resistance gene; bar) and GFP expression (green fluorescent protein). We found over 20 mutants that were phenotypically stable over five subcultures. Southern analysis showed that some of the mutants had single insertions of the T-DNA fragment from the binary vector. Analysis of the regions flanking the point of vector insertion into the M. anisopliae genome, using YADE (Y-shaped adaptor dependent extention), revealed four genes with significant homologies to conidiation and colony phenotype genes from other filamentous fungi. We view the ability to characterize genes involved in conidiation, and their potential control using inducible promoters, as essential in order to prevent the reproduction and dissemination of genetically altered strains in the field.

Contributed paper. Wednesday, 8:15. **101**

Directed adaptation of *Metarhizium anisopliae* to cockroach cuticle

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Metarhizium anisopliae is a cosmopolitan broad host range arthropod pathogen. Strains of *M. anisopliae* have been selected for control of insects and other arthropods that act as disease vectors including mosquitoes and ticks, crops pests such as whiteflies and borers, and ecologically hazardous, invading pests such as fire ants and termites. The major strategy for finding fungal strains with increased virulence towards or targeting of specific hosts has been to collect and isolate field specimens from infected insects. Using an automated continuous culture machine that actively selects for fast growing variants, *M. anisopliae* strain 2575 was adapted for growth on cockroach cuticle. The rate of growth on cockroach cuticle increased 2-fold after 11-cycles of the machine. Within 17 cycles growth of the *M. anisopliae* within the flexible tubing growth chamber appeared to proceed almost exclusively by microcycle conidiation. Isolate EVG 0525 (8 cycles) when plated on Potato Dextrose (PD) agar sporulated very poorly. In order to increase and/or maintain robustly sporulating isolates the continuous culture machine was modified to allow for cycles of sporulation during the selection scheme. These data provide preliminary evidence that this technology can be used to adapt fungal strains to host nutrient sources.

Contributed paper. Wednesday, 8:30. **102**

The effect of tick species and stages on the pre-penetration steps of the entomopathogenic fungi, *Metarhizium anisopliae*

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Ticks are high-efficiency vectors of vertebrate pathogens making them important pests of people and pets as well as domestic and wild animals. Though ticks feed only on vertebrates, most of their life cycle is spent on ground. Twenty fungi species attack ticks in nature with infection variation attributed to tick stage and species along with ecological niche conditions. Utilizing novel techniques, here we demonstrate that the amount of *M. anisopliae* var. *anisopliae* conidia adhering to tick cuticle is a dose-response process that is directly correlated with tick mortality. Conversly, the amount of conidia of the low virulent isolate *M. an.* var. *acridum* also adheres to ticks in correlation to the infecting dose but with no correlation to tick mortality. Cuticular lipids from different tick species and stages stimulate conidia germination. However, formation of *M. an. an.* appressorium, largely depends on the tick susceptibility to the fungus. The germination of *M. an. ac.* conidia is hardly stimulated by lipids extracted from any of the tick species tested and none of the extracts stimulate the formation of appressorium. While the adhesion of conidia to ticks is mainly an abiotic process, the cuticular lipids play an important role in the anti-fungal protection or in initiating the virulence steps of ticks.

Contributed paper. Wednesday, 8:45. **103**

A proteomic approach to the identification of proteins differentially expressed in the conidia and mycelium of the entomopathogenic fungus *Metarhizium anisopliae*

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Conidia are specialized structures of filamentous fungi responsible for the reproduction, dispersal and environmental persistence of these microorganisms. In pathogenic species, the conidia are also involved in host recognition and infection. Conidia present biochemical, physiological and morphological differences in relation to the mycelium that are largely due to differences in the sets of enzymes and structural proteins present in the two developmental stages. We used a proteomic approach to isolate and identify proteins present in the conidia and mycelium of the ARSEF 324 strain of *M. anisopliae* var. *acridum*. Proteins present in conidial and mycelial extracts were separated by two-dimensional electrophoresis and identified by MALDI–MS/MS. The results showed that there is a great difference between the sets of proteins present in the mycelium and the conidia. Approximately 901 proteins and/or isoforms were isolated from conidia, and there were 917 from mycelium. Only 481 were common to the two structures. Among the proteins found exclusively and more abundantly in conidia were an HSP 30, a 6-phosphogluconarate dehydrogenase, the allergen Alt a 7, a predicted vacuolar protease A, and a predicted mitochondrial peroxyredoxin. In contrast, mycelium expressed specific stage proteins of primary metabolism, such as a citrate synthase and a ketol-acid reductoisomerase (a mitochondrial precursor).

Contributed paper. Wednesday, 9:00. **104**

Transcript analysis of the entomopathogen *Beauveria bassiana* during the infection process on the coffee berry borer

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To understand the infection process of the entomopathogen *Beauveria bassiana* on the Coffee Berry Borer (CBB) *Hypothenemus hampei*, full length cDNAs were obtained from mycelium of the strain Bb9205 growing for 4h in a minimal medium plus 10% w/v CBB and from spores growing for 24h in minimal medium plus CBB. A differential library was also constructed by subtractive hybridization of mRNAs from Bb9205 growing in SDB (driver) and in minimal medium plus CBB (tester). A total of 2300 clones sequenced from each of the full length libraries, plus 250 clones produced by subtractive hybridization, were checked for quality and assembled into 2401 unigenes (598 contigs and 1803 singlentons) with an average size of 690 bp. Annotation against

GenBank resulted in 1% of the unigenes corresponding to ribosomal sequences, 44% without significant matches (associated to large contig families), 44% related to hypothetical proteins of unknown function, and 11% (269) significantly similar to proteins with known function. Annotated sequences with relative highly expression and reportedly connected to pathogenicity are related to heat shock response, active oxygen metabolism, antibiotic production and protease activity. Detailed analysis of the expression patterns of these transcripts is required to determine their role and importance in virulence. Co-financed by the Colombian Ministry of Agriculture and Rural development.

Contributed paper. Wednesday, 9:15. **105 STU**

Alkane degradation by *Beauveria bassiana*: Gene expression analysis of cytochrome P450 monooxigenases

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The insect waxy layer, comprised of a complex mixture of lipids that include abundant amounts of straight-chain and methyl-branched, saturated and unsaturated hydrocarbons, represents the first barrier to infection by entomopathogenic fungi. Degradation of these hydrocarbons is presumed to occur via oxidation by cytochrome P450 enzyme systems. Seven gene fragments displaying high homology to cytochrome P450 alkane and/or aromatic oxidizing enzymes as well as a putative lipid-carrier protein were identified in a *B. bassiana* expressed sequence tagged (EST) collection. Full-length sequences for each gene were isolated by 5' and 3'-rapid amplification of cDNA ends (RACE). Expression analysis of the genes by real-time RT PCR using fungal cells grown on *n*-hexadecane (C16), *n*-eicosane (C20), or *n*-octacosane (C28) revealed overlapping but differential expression of subsets of the isolated P450 genes. These data indicate that *B. bassiana* is likely to contain multiple hydrocarbon degradative pathways with overlapping substrate specificities.

Contributed paper. Wednesday, 9:30. **106 STU**

May *Beauveria bassiana* secreted proteins be virulence factors?

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Fungal virulence has been mostly associated with cuticle-degrading enzymes which can be regulated depending on nutrient conditions. However, few studies relate fungal virulence to fungal insecticidal secreted proteins. We report how the ability of secreting these proteins may be linked to conidial virulence which can be affected by nutrient factors. In this study we evaluated: (1) the virulence of the conidia of 4 *Beauveria bassiana* isolates (EABb 01/110-su, EABb 01/103-su, EABb 01/12-su and EABb 01/88-su) grown on 4 different media; Malt Extract Agar (MEA), Rice (R), Sabouroad Glucose Agar (SGA) and Infected 4th instar *Galleria mellonella* Larvae (IL), and (2) the toxicity of Fungal Insecticidal Proteins (FIP) obtained from those conidia when inoculating in Adamek's liquid medium. Conidial suspensions were obtained from the 4 media, assessed on *G. mellonella* larvae and used for production of FIP which were injected in healthy *G. mellonella* larvae. In all isolates, probit analysis and parallelism test showed that conidia from IL were by far the most virulent, followed by conidia from SGA, R and MEA. Toxicity of FIP showed the same trend of conidial suspensions although relative potencies are not that different. Assay with hemolymph from larvae, which were previously injected with conidia of these *B. bassiana* isolates grown on MEA and IL, showed that: (1) EABb 01/110-su isolate does not produce FIP *in vivo*, only EABb 01/103-su, EABb 01/12-su and EABb 01/88-su isolates do, this suggests that the virulence of EABb 01/110-su isolate is due to cuticle-degrading enzymes which are toxic when injected;(2) hemolymph from injected larvae with conidia grown on MEA are less toxic than that from injected larvae with conidia grown on IL. *In vitro* and *in vivo* studies suggest that nutrient conditions influence conidial virulence of EABb 01/103-su, EABb 01/12-su and EABb 01/88-su isolates by enhancing secretion of FIP after host infection.

Contributed paper. Wednesday, 9:45. **107**

Live cell imaging of endocytosis and membrane properties of *Beauveria bassiana in vitro* and hemolymph derived cells

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Beauveria bassiana produces several distinct single cell types that include aerial conidia, *in vitro* blastospores, and submerged conidia. Under appropriate nutrient conditions these cells can elaborate germ tubes that form hyphae, which in turn lead to the formation of a fungal mycelium. In addition, *B. bassiana* displays a dimorphic transition, producing specific *in vivo* yeast-like hyphal bodies during growth in the arthropod hemolymph. Furthermore, *in vitro*, in nutrient media composed of proteose peptone containing either lactose or trehalose, cells similar in morphology to the *in vivo* hyphal bodies can be produced. The amphiphilic styryl dye FM4-64 was used to investigate internalization and morphologic features of the *in vitro* and *in vivo* derived *B. bassiana* cells. Both *in vitro* blastospores and submerged conidia displayed a punctate pattern of internal labeling, whereas aerial conidia failed to internalize the dye under the conditions tested. FM4-64 was also taken up into both apical and subapical compartments of living hyphae in a time-dependent manner with clearly observable vesicle labeling. The effects of various metabolic and endocytic inhibitors including azide/fluoride, cyanide, lactrunculin B, and cytochalisin D were examined in the germlings. In contrast to the *in vitro* cells, fungal cells derived from infected insect hemolymph (*in vivo* cells) displayed weak and differential FM4-64 uptake. These cells known as *in vivo* blastospores or hyphal bodies were much more recalcitrant to dye uptake, with weak membrane and internal straining visible. FM4-64 uptake in the *in vitro* lactose/trehalose derived cells was also investigated. These results suggest active uptake by different developmental stages of *B. bassiana* and differential cell wall remodeling during *in vivo* cell propagation of the fungus in target hosts.

CONTRIBUTED PAPERS Wednesday, 8:00-9:45

VIRUSES 3

Contributed paper. Wednesday, 8:00. **108**

Deletion of the *egt* gene reduces within-host competitive fitness

Mark Zwart[1]; Wopke van der Werf[2]; Monique van Oers[1]; Lia Hemerik[3]; Jan van der Lent[1]; Arjan G. M. de Visser[4]; Just M. Vlak[1]; Jenny S. Cory[5]
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Given the high genetic diversity of insect baculoviruses co-infections are likely to be common in natural populations. The baculovirus life cycle comprises two main components, infection and spread in the host (within-host dynamics) and transmission to naïve hosts (between host transmission); most mixed infection studies have targeted the 'between host' component. We investigated within-host mixed infections using an AcMNPV clone lacking an effective ecdysteroid UDP glucosyl transferase (*egt*) gene. It is well established that deletion of *egt* results in a more rapid kill, with resultingly lower occlusion body (OB) yield. This is assumed to enhance between host transmission because more inoculum is released into the environment. However, whether the possession of this gene is likely to have a cost or a benefit for within-host infection is not known. The results indicate that dual genotype infection parameters can be predicted from single infections, in a qualitative sense, for speed of kill, and to a lesser extent, virus yield. Considerable variation in the genotype ratio was observed between individual larvae; possibly as a result of a small number of virions initiating infection. Longer term passage experiments showed that there was selection for the parent wild type genotype over the *egt* deletion strain.

Contributed paper. Wednesday, 8:15. **109 STU**

Characterization of climbing behavior gene in recombinant baculoviruses

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Wild gypsy moth larvae climb only prior to molting and wander prior to pupation. However, larvae infected with *Lymantria dispar* nucleopolyhedrosis virus (LdNPV) climb high on host trees several days before death, remaining there until succumbing to virus. We hypothesized that the *egt* gene (ecdysteriod UDP-glucosyltransferase) affects climbing prior to death. Behavioral tests using constructs of LdNPV containing *egt* or with *egt* deleted demonstrated that larvae infected with the deletion constructs did not climb prior to death, while larvae infected with wildtype virus or with a transgenic reporter constructs containing *egt* died at elevated positions. We examined two recombinant baculoviruses expressing lac Z, both purportedly containing an intact *egt* gene. One recombinant (323c) induced climbing behavior in larvae similar to wild type infected insects, while the other (7H5) did not. Amplification of the *egt* gene was successful for 323c, but not 7H5. Restriction digests showed a deletion in the *egt* gene in 7H5. qRT-PCR indicated elevated *egt* gene expression in larvae infected with the wild type and 323c, while *egt* expression was suppressed in larvae infected with 7H5. These results confirm observations that insects infected with LdNPV lacking intact *egt* gene do not seek elevated positions prior to death.

Contributed paper. Wednesday, 8:30. **110 STU**

Conservation of DNA photolyase genes in plusiine nucleopolyhedroviruses

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DNA photolyase genes (*phr*) encode photoreactive enzymes, which are involved in the repair of UV-damaged DNA. Cyclobutane pyrimidine dimer (CPD) specific photolyase genes are present in nucleopolyhedroviruses isolated from *Chrysodeixis chalcites* (ChchNPV) and *Trichoplusia ni* (TnSNPV) insects belonging to the *Plusiinae subfamily* (Noctuidae). To better understand the occurrence and evolution of these genes in baculoviruses, we investigated their possible conservation in other group II NPVs, which infect plusiine insects. A PCR based strategy using degenerate *phr*-specific primers was designed and validated to detect and analyze possible photolyase genes. Six additional *Plusiinae*-infecting NPVs were analyzed and all but one contained one or more *phr*-like sequences. *Thysanoplusia orichalcea* NPV A28-1 appeared to be a group I NPV, and did not contain a *phr* homologue. Phylogenetic analysis revealed that all photolyase genes of the tested *Plusiinae*-infecting baculoviruses group in a single clade. Moreover, the phylogeny of the polyhedrin sequences of these viruses confirmed that the analyzed viruses also formed a single clade in group II NPVs. We hypothesize that all plusiine group II NPVs contain one or more photolyase genes and that these have a common ancestor. The correlation between baculoviruses having *phr* genes and the behaviour of their plusiine hosts will be discussed.

Contributed paper. Wednesday, 8:45. **111 STU**

***Chrysodeixis chalcites* nucleopolyhedrovirus encodes an active DNA photolyase**

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UV-radiation induces two types of lesions in DNA: cyclobutane pyrimidine dimers [CPD] and (6-4) photoproducts. These lesions can be repaired by specific DNA photolyases, which occur in all organisms except placental mammals. DNA photolyases are active under the influence of blue light. On the basis of amino acid homology two classes of CPD photolyases can be distinguished. Recently, class II CPD photolyase (*phr*) genes have been identified in plusiine-infecting group II nucleopolyhedroviruses (NPVs). In the *Chrysodeixis chalcites* NPV genome two putative *phr* genes are present. Expression of Cc-*phr2*, but not Cc-*phr1*, was able to complement a photolyase deficiency in *Escherichia coli*, indicating that Cc-*phr*2 encodes an active photolyase. To further characterize this photolyase, Cc-*phr2* was overexpressed in *E. coli* and the resulting photolyase was purified. Spectral measurements indicated the presence of FAD as co-factor, but a second chromophore appeared to be absent. I*n vitro, r*ecombinant Cc-phr2 photolyase specifically bound F0 (8-hydroxy-7,8-didemethyl-5-deazariboflavin), a rare antenna chromophore in eukaryotes. After reconstitution of the photolyase, FAD and F0 were present in approximately equimolar amounts. The F0 chromophore is functionally active in reconstituted Cc-phr2 photolyase as judged from the increase in the *in vitro* DNA repair activity. This study

demonstrates for the first time that a functional photolyase is encoded by an insect virus and this may have implications for baculovirus applications in insect biocontrol.

Contributed paper. Wednesday, 9:00. **112 STU**

Anti-viral defenses in gypsy moth larvae: Evidence for the importance of immune responses within the host

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Gypsy moth (*Lymantria dispar*) larvae show intrastadial developmental resistance (IDR) to the baculovirus *Lymantria dispar* multiple nucleopolyhedrovirus (LdMNPV); newly molted larvae are 40-50% more susceptible to a given dose of virus than those inoculated 48-72 hours post-molt. We hypothesized that there are differences in the immune responses between the larval ages, and they contribute to IDR. We have identified several possible immune processes that may be anti-viral defenses, such as cellular encapsulation, phenoloxidase (PO) mediated melanization, and apoptosis of infected cells. To examine cellular encapsulation, we inserted infected tissue into larvae of different ages and observed the extent of the immune response to the inserts. We also inoculated both susceptible and resistant-aged larvae intrahemocoelically and measured the PO activity in the hemolymph periodically following the inoculation. Finally, we established if apoptosis was occurring in infected tissues by assaying infected trachea for apoptotic cells. From our results, resistant-aged larvae show a greater cellular immune response and exhibit higher PO activity than susceptible-aged larvae. Apoptosis occurs readily in infected trachea of both ages of larvae, but the impact on infection appears to be greater in resistant-aged larvae. These findings indicate the importance of the immune system in IDR, and in the future we will continue to define the mechanisms behind these anti-viral defenses.

Contributed paper. Wednesday, 9:15. **113**

Baculovirus infection of immunosuppressed *S. littoralis* as a tool to study the lepidopteran anti-viral response

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Spodoptera littoralis, a Mediterranean insect pest, is highly resistant to infection by the baculovirus AcMNPV. Oral infection of 2nd instar larvae require high viral dose to cause 50 % mortality of the insect population. Analysis of infected larvae showed that the insect immune system reacts to the orally-acquired viral particles by encapsulating them in the insect midgut, blocking the propagation of the virus. Targeted immunosuppression of the host, achieved by engineering orally-infectious recombinant AcMNPV bearing the polydnavirus genes *VHv1.1* and *P-vank1* from the *cys*-motif and *vankyrin* families, resulted in enhanced viral pathogenicity. Infection of *S. littoralis* with GFP-tagged versions of the above recombinant baculoviruses showed that their enhanced infectivity was due to successful propagation of the viral particles through the insect body. Moreover, *VHv1.1* and *P-vank1* were able to increase AcMNPV pathogenicity in a cooperative manner. These and recent findings suggest that different molecular pathways are involved in implementing the anti-viral response in Lepidopterans.

Contributed paper. Wednesday, 9:30. **114**

An Ac*M*NPV *fgf* knockout mutant exhibits a defect in systemic infection of *Trichoplusia ni* larvae

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Fibroblast growth factors (FGFs) are a family of growth factors that have been shown to be involved in cell differentiation and proliferation. To date, all baculoviruses that encode at least one *fgf* homolog (*vfgf*) establish systemic infections in their hosts. However, known baculoviruses that do not encode *fgf* homologs are limited to midgut epithelial cell infection. We have previously shown that an Ac*M*NPV *vfgf* knockout virus does not have any obvious replication defects in cell culture, but causes slower mortality in *Spodoptera frugiperda* and *T. ni* larvae. We previously hypothesized that *vfgf* may facilitate the systemic spread of the virus *in vivo*. In this study, we compared the infection of tissues in *T. ni* larvae with viruses containing or lacking Ac*M*NPV *vfgf*. We found that there is a defect in systemic infection following oral infection of *T. ni* larvae with the *vfgf* knockout mutant. The defect is not obvious in all tissues if the virus is delivered intrahemocoelically. These results suggest that *vfgf* aids in the establishment of efficient Ac*M*NPV infection in *T. ni* larvae.

SYMPOSIUM (Div. of Nematodes) Wednesday, 10:30-12:30

Entomopathogenic Nematode Application Technology in IPM

Symposium. Wednesday, 10:30. **115**

Current status in application technology

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Nematodes are expensive active ingredients and a reduction in dosage by improved application techniques is needed to open new markets. In turf, it is now common practice to use specific tensides to improve the penetration of water and nematodes through dry patches of the thatch layer. If available, nematode application via the irrigation system is one of the best options since they are delivered to the moist soil parts where the pest insects are most likely to feed. Initial supervision and training is however often required to establish this application regime under the specific conditions of a certain grower. Nematode products against cockroaches and woodlice are applied in “attract and kill”-stations. Stations like these might be suitable for other pests, as well. Since the pest encounters very high doses in the bait station, even pests which were considered unsusceptible can probably be killed with nematodes using this approach. A targeted application to the plant can be achieved by applying nematode agglomerates - as granules or infected insects - during sowing or planting, but is not yet implemented in practice. Abovegrond applications in non-protected crops (apples) have recently been established.

Symposium. Wednesday, 11:00. **116**

Cadaver application

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In the last two decades, entomopathogenic nematodes (EPNs) were mainly applied against insect pests in aqueous suspension to the soil; however, this application method presents limitations. In orchards, and in other cases where the insect pests are concentrated, EPNs can be successfully applied as infected host cadavers. Host cadavers, with infective juveniles (IJs) emerging over 2-14 days, may act as effective slow release capsules. Several studies have shown that IJs emerging from infected cadavers show superior migratory capability, infectivity, and persistence in soil when compared to IJs applied in aqueous suspension. This methodology has been efficacious against the guava weevil, *Conotrachelus psidii*, a major pest of guava in Brazil, as well as several other weevil pests in potted plants. In guava, IJs from the cadavers were detected for 6 weeks after application in the field, but decreased thereafter. Recently the temporal-spatial soil pattern of *Heterorhabditis baujardi* LPP7 IJs was established when applied as host cadavers under field conditions, starting from a point source, using two different host cadaver concentrations. The problems of achieving successful control using this strategy are discussed; the goal is to minimize costs and increase the success of IPM programmes for the growers.

Symposium. Wednesday, 11:30. **117**

Above ground and cryptic habitats application

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Using entomopathogenic nematodes in IPM programmes is common, even in intensive Spanish greenhouse production. Delivery of viable EPN to the target is critical for above ground application, where drenching techniques are not feasible. Growers want to use existing pesticide application equipment, which involves large tanks, recirculation and filtering systems with mechanical pumps, some of which can reduce EPN viability. EPN have been shown to be particularly resilient when used with pesticide application equipment. However, for an application of EPN to a crop to be successful they must be delivered to the correct location within the crop canopy, to allow them to locate the pest on the plant. In the warm dry conditions of southern Europe this can be a problem, as the carrier water evaporates rapidly. Although a number of EPN have been shown to successfully control a wide range of arthropods under laboratory conditions, the next step to deployment in the field can often fail. The problems of achieving successful application and delivery strategies for optimising EPN survival and pest control will be discussed, including targeting and timing the application to minimise water volumes required, a key factor in developing successful and economical IPM programmes for the grower.

Symposium. Wednesday, 12:00. **118**

Enhancing post-application survival of entomopathogenic nematodes

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The post application survival of infective juveniles of Steinernema and Heterorhabditis species will depend on the environment in which the infective juveniles (IJs) are applied, available moisture and the ambient temperature at the time of application. Restricting application to soil and cryptic habitats when temperatures are favorable for the nematode species will enhance activity and survival of IJs. Moisture can be maintained in these habitats in a variety of ways including pre- post application irrigation, use of humectants and mulches. Mulch that is wetted before and after application has provided one of the best methods for maximizing survival and insecticidal activity for control of a variety of insects in soil and leaf litter habitats. Moisture maintenance in other cryptic habitats is more challenging. For example, the treatment of fruit bins to control diapausing codling moth was achieved by dunking the bins in a suspension of IJs to which a wetting agent and a humectant were added.

CONTRIBUTED PAPERS Wednesday, 10:30-12:15

BACTERIA 2

Contributed paper. Wednesday, 10:30. **119**

A novel gene cluster encoding an insect toxin in plant-associated strains of *Pseudomonas fluorescens*

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Pseudomonas fluorescens CHA0 and the related strain Pf-5 are well-characterized rhizosphere bacteria that have the capacity to protect crop plants from fungal root diseases, mainly by releasing a variety of exoproducts that are toxic to plant pathogenic fungi. Here, we report that the two plant-beneficial pseudomonads exhibit potent insecticidal activity. Anti-insect activity is linked to a novel genomic locus encoding a large protein toxin termed Fit (for *P. fluorescens* insecticidal toxin) that is related to the insect toxin Mcf (Makes caterpillars floppy) of the entomopathogen *Photorhabdus luminescens*, a mutualist of entomopathogenic nematodes. When injected into the hemocoel, even low doses of *P. fluorescens* CHA0 or Pf-5 killed larvae of the tobacco hornworm *Manduca sexta* and the greater wax moth *Galleria mellonella*. By contrast, mutants of CHA0 or Pf-5 with deletions in the Fit toxin gene were significantly less virulent to the larvae. When expressed from an inducible promoter in a non-toxic *Escherichia coli* host, the Fit toxin gene was sufficient to render the bacterium toxic to both insect hosts. Our findings establish the Fit gene products of *P. fluorescens* CHA0 and Pf-5 as potent insect toxins that define previously unappreciated anti-insect properties of these plant-colonizing bacteria.

Contributed paper. Wednesday, 10:45. **120 STU**

Functional characterisation of a cell cycle inhibiting factor (CIF) in the entomopathogenic bacteria *Photorhabdus*

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Photorhabdus is an entomopathogenic bacterium symbiotically associated with soil nematodes belonging to the genus *Heterorhabditis*. The genomes of *Photorhabdus luminescens* and *asymbiotica* (emergent human pathogen) contain a homologous to the cyclomodulin gene *cif* (Cycle Inhibiting Factor) of enteropathogenic *Escherichia coli*. In *E. coli*, Cif is translocated into the host cell by the type III secretion system (TTSS) and blocks cell cycle transition. In this study, we investigated the distribution and genetic environment of *cif* in different *Photorhabdus* species. *cif* is present in most of the *P. luminescens* and *P. asymbiotica* species. In contrast, it is likely that *P. temperata* strains have no *cif* homologue. Analysis of the genomic regions surrounding *cif* in *Photorhabdus* revealed that it is located in a lamboid prophage environment as *E. coli-cif*. We observed that the introduction of *Photorhabdus*-Cif in HeLa cell induces cycle arrest and the formation of stress fibres, a phenotype already described for *E. coli*-Cif. This suggests that *Photorhabdus*-Cif is a cyclomodulin. We are currently investigating the effect of *Photorhabdus*-Cif on insect cells. In vitro and in vivo studies suggest that *Photorhabdus*-Cif secretion is TTSS independent. Finally, we will discuss the involvement of Cif in the *Photorhabdus/ Heterorhabditis* symbiosis.

Contributed paper. Wednesday, 11:00. **121 STU**

Secondary lipid A acylation and extrusion by efflux pumps are two potential mechanisms of resistance to anti-microbial peptides in the entomopathogenic bacterium *Photorhabdus luminescens*

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Photorhabdus luminescens is a Gram-negative bacterium that is pathogenic to insects while also maintaining a mutualistic relationship with the entomopathogenic nematode *Heterorhabditis. Photorhabdus* genus has a natural resistance to many Anti-Microbial Peptides (AMPs). This is likely the reason why the bacteria regurgitated by the infective nematodes into the insect hemolymph are able to multiply and kill phytophagous *Spodoptera* larvae within 24 to 48 hours after infection. Here, we describe in *P. luminescens* TT01 the role of the *msbB* gene (*lpxM*) in resistance to cationic AMPs. *msbB* encodes an enzyme responsible for late secondary acylation of immature lipid A molecules. On the other hand, we have constructed a *P. luminescens* genomic DNA library in the susceptible *E. coli* strain XLI Blue and screened for clones with increased resistance to AMPs. A clone harboring *mdtC* and *baeS* genes confers resistance to polymyxin B and polymyxin E and cecropin A. MdtC is a transmembrane homomultimer of the multidrug transporter MdtABC. BaeS is the sensor of the two component regulatory system *baeSR* which activates several drugs efflux pumps in bacteria. Our findings suggest that the *msbB* gene and the *mdtABC/baeSR* operons are potential candidates involved in *P. luminescens* resistance to *Spodoptera's* AMPs and eventually in insect pathogenicity.

Contributed paper. Wednesday, 11:15. **122 STU**

Structural studies of toxin complexes

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The Toxin complex (Tc) genes were first identified in the insect pathogen *Photorhabdus luminescens* strain W14. Thes high molecular weight proteins (~1MDa) are orally toxic to insects. They are encoded by 4 loci; *tca, tcb, tcc* and *tcd*, the genes within these loci labeled according to their order e.g. *tcdA, tcbB, tccC*. Significant homology is observed between the loci and previous work has shown all three components, the *tcdA*-like [A], the *tcbB*-like [B] and the *tccC*-like [C] genes are required for full toxicity. Preliminary analysis of heterologously expressed *P. luminescens* TcdA [A] and TcdB [B] by transmission electron microscopy (TEM) shows a "lollipop" structure. Addition of TccC [C] does not affect the physical appearance of the TEM complex, however, it is required for toxicity. Subsequently, *X. nematophila* XptA1 [A], a homologue of *P. luminescens* TcdA [A] was determined to be a 1.15MDa complex, consistent with a tetramer by ultracentrifugation. TEM revealed a 23Å bottle-like structure consisting of a tetramer held together in a supramolecular ring, with one end narrower than the other, consistent with [A] contributing to the "head" of the lollipop structure and suggests [B] is the "stick". Here I present biophysical and strucural data of [BC].

Contributed paper. Wednesday, 11:30. **123 STU**

Interaction between Cry1Ab oligomer and their receptors alkaline phosphatase and aminopeptidase-N from *Manduca sexta*

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The Cry toxins produced by *Bacillus thuringiensis* (Bt) are used worldwide as an effective biological control agent for many species of insects including agricultural and forest pests, and several vectors of human and animal diseases. The major threat to the use of Cry toxins is the appearance of insect resistance. The most frequent mechanisms of resistance to Cry toxins are defects on toxin-receptor interactions. For Cry1A toxins (lepidopteran specific toxins), at least four different protein-receptors have been described; a cadherin-like protein (Cader), a glycosylphosphatidyl-inositol (GPI)-anchored aminopeptidase-N (APN), a GPI-anchored alkaline phosphatase (ALP) and a 270 kDa glycoconjugate. In this work we characterize the interaction of oligomer of Cry1Ab toxin with APN and ALP. The receptors were purified from *Manduca sexta* midgut by chromatography of ion exchange and affinity respectively. First, we evaluated the interaction of the Cry1Ab oligomer and monomer with aminopeptidase and alkaline phosphatase. Then, we perform competition experiments using synthetic peptides corresponding with some regions of Domain II and III as competitors. Our results support the Bravo´s model where the Cry toxins change the conformation after oligomerization and interaction with a second receptor is necessary for the toxicity.

Contributed paper. Wednesday, 11:45. **124 STU**

Cry1Ab oligomeric structure elucidated by transmission electron microscopy

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Bacillus thuringiensis (Bt) gram-positive bacteria produce insecticidal toxins (Cry) during sporulation phase. These are toxic to different insect species and nematodes. Cry toxins primary action is to lyse midgut epithelial cells in their specific targets. Nevertheless their mode of action is not completely understood. In our group, we support the hypothesis that Cry toxins are pore forming toxins. We described the sequential interaction of Cry1A toxins with receptors, cadherin and APN, and the formation of an oligomeric pre-pore structure that is important for pore formation and toxicity. Also we demonstrated that Cry1Ab oligomer is an obligate intermediate in toxicity against the target insect. We isolated mutants R99E and Y107E located in helix a-3 affected in oligomer formation. These two mutants were perfectly processed and bound to Bt-R1 receptor with similar affinity as wild type toxin (K_D=8nM). However, they were severely affected in their toxicity (LC_{50}>2000 ng/cm^2) and pore forming activity analyzed in black lipid bilayers. These mutants didn't form oligomeric structures, supporting our hypothesis that Cry1Ab oligomer is necessary for Cry1Ab toxicity. Until the moment we don't have any information about Cry1A oligomeric structure. Recently, it was reported the construction of 2D crystals of Cry4Ba oligomer that were analyzed by transmission electron microscopy (TEM). In that work, it was showed that Cry4Ba oligomer is a trimer, but information about interactions between monomers inside the trimeric structure was missing. The goal of this work was the construction of Cry1Ab oligomeric 2D crystals using protein reconstitution technique. These 2D crystals were negative stained and analyzed by TEM.

Contributed paper. Wednesday, 12:00. **125**

Effects of amino acid substitutions in a loop connecting beta-6 and beta-7 of a cytolytic toxin from *Bacillus thuringiensis*

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Cyt2Aa2 is a cytolytic and mosquito-larvicidal toxin produced by *Bacillus thuringiensis* subsp. *darmstadiensis*. The toxin gene has been cloned and expressed in *E. coli* as protoxin inclusions. The inclusions are solubilized in the alkaline condition of the mosquito larval gut and activated by gut proteases. The activated toxin will bind to the epithelium gut cell membrane, oligomerize and form pores leading to cell lysis. To investigate amino acid residues that might play an important role during conformational changes upon toxin binding to the membrane, seven single-amino acid substitutions in the beta6-beta7 loop were performed (S194A, V197A, K199A, Q200A, K201A, F204A and T206A). Replacement by alanine at Lys-199, Lys-201 and Thr-206 affected protein folding and the mutated proteins were degraded after protease activation. Other mutants yielded a similar product to the wild type and should adopt a similar 3D structure. Mosquito-larvicidal activity was significantly improved for mutants S194A, Q200A and F204A but decreased for K199A and T206A. Hemolytic and mosquito-larvicidal activities were completely lost for the mutant V197A although this mutant could maintain a similar structure to the wild type toxin. Results suggested that Val-197 might play a critical role during conformational changes upon binding to the membrane.

CONTRIBUTED PAPERS Wednesday, 10:30-12:15

MICROBIAL CONTROL 2

Contributed paper. Wednesday, 10:30. **126**

Beauveria bassiana* as an artificial endophyte in tissue-cultured banana (*Musa* spp.) plants: A novel way to combat the banana weevil *Cosmopolites sordidus

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Studies have revealed great potential of *Beauveria bassiana* for use against the banana weevil, *Cosmopolites sordidus*, in banana (*Musa* spp.). However, impractical field delivery methods and high costs associated with application prevent its use and commercialization in banana fields. Our research has revealed that *B. bassiana* can colonize the internal banana tissues for at least four months when tissue-cultured plantlets are dipped in a spore suspension. *Beauveria bassiana* colonization was not dependent on banana cultivar and even when elevated *B. bassiana* doses were used, plant growth was not negatively affected. In a set of screenhouse experiments, *B. bassiana*-enhanced plants inflicted 23.5-88.9% larval mortality and the presence of endophytic *B. bassiana* inside treated plants led to a reduction in larval damage of >50%. Application of *B. bassiana* as an artificial endophyte inside banana plants could circumvent bottlenecks associated with its application as a conventional biopesticide, because i) it kills the damaging stages inside the plant, ii) it is protected from adverse biotic and abiotic factors, iii) little inoculum is required, drastically reducing its cost, and iv) farmers do not need to apply the biological control organism themselves, as the technology is easily transferable to a commercial tissue culture producer.

Contributed paper. Wednesday, 10:45. **127**

Assessing the field persistence of introduced *Beauveria bassiana* GHA for emerald ash borer control by bioassay, culture on selective medium and real-time PCR

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Determining field persistence of mycoinsecticides is essential in order to develop effective and economical application strategies, including specifically the timing and frequency of spray applications. In this study we compared three methods of evaluating the persistence of Beauveria bassiana strain GHA applied for control of the emerald ash borer, an invasive pest attacking ash trees, Fraxinus spp., in North America. Fungal inocula present on ash bark and leaves and in soil sampled nearby, collected at 0, 7 and 14 days after spraying, were quantified by use of molecular (real-time PCR assay) and culture-based methods (semi-selective wheat germ dodine

agar). In addition, we conducted bioassays using emerald ash borer adults to determine whether the level of inoculum persisting in the field was sufficient to affect their survival on treated foliage or bark. Persistence of sufficient conidia on either substrate may make pre-emergent sprays a practical means to target adults during emergence, pre-oviposition feeding, or oviposition. Results of assays during two seasons will be presented.

Cancelled: Contributed paper. **128**

Contributed paper. Wednesday, 11:00. **129**

Improving efficacy of *Beauveria bassiana* foliar treatments against Colorado potato beetle via manipulation of spray-application parameters.

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Effects of hydraulic spray pressure and sprayer configuration on efficacy of foliar applications of *B. bassiana* against *Leptinotarsa decemlineata* larvae were evaluated during four field seasons. Treatments were applied to small plots using a tractor-mounted sprayer with nozzles mounted on swivels on short drop tubes spaced 21.5 cm apart. Nozzles (four per potato row) were positioned at canopy height and directed forward and downward at a 45º angle to the ground. The sprayer was alternatively configured with nozzles mounted on double swivels on drop tubes centered between the rows. Nozzles (two per row) were positioned at ca. mid crop height, directed perpendicular to the row, and angled upward at an 85º angle to the ground. The sprayer was operated at a ground speed of 4.8 km/h. Hollow-cone nozzles of four different sizes were selected to deliver a constant volume of ca. 467 L/ha at four different pressures: four nozzles/row @ 345 kPa (configuration A), 690 kPa (B), and 2,758 kPa (C); 2 nozzles/row @ 621 kPa (D). Conidia of *B. bassiana* strain GHA formulated as a wettable powder were applied 3–4 times/season at 3–5-day intervals. Each application was made at the rate of 2.5 x 10^{13} conidia/ha; treatments included untreated and spray-carrier controls. Significantly greater larval mortality resulted from the C vs. A and B configurations (34 vs. 12 and 15%, respectively). Results from the D configuration (29% mortality) were equivalent to C. The C and D configurations also produced greater reductions in second-generation adult populations than the A and B configurations (86 and 87% vs. 75 and 81%, respectively); however, treatment differences were not significant. Coverslips placed in the upper-center crop canopy revealed that the A, B, C, and D configurations delivered statistically equivalent total numbers of conidia to the targeted plants (1019, 1007, 1110, and 1244 conidia/mm^2, respectively); however, the 4 nozzle-high pressure spray (C) delivered a significantly greater percentage of the total conidia to coverslips attached to abaxial surfaces of potato foliage than the other three sprays (27 vs. 11–13%). Results suggest that the efficacious delivery of conidia by the 2-nozzle/row configuration was not accurately measured by the above-described protocol (foliage at the lateral edges of the crop canopy may have shielded the centrally-located coverslips). This study indicates that modifying spray parameters can increase efficacy of biopesticide sprays against potato beetles; however, the increases we observed were small and may not justify the added costs associated with the required modifications.

Contributed paper. Wednesday, 11:15. **130 STU**

Effect of storage conditions on the pathogenicity of entomopathogenic fungi *Beauveria bassiana* to control whitefly *Bemisia tabaci*

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Whiteflies are one of the most important arthropod pests of greenhouse and field crops, *Bemisia tabaci* occurring mostly in tropical and subtropical climates. The entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin displays a broad host range and is able to target about 200 species of diverse arthropod species. Shelf-life is a crucial factor for the acceptance of microbial insecticide by growers and public. Additives enhance the storage potential, spore germination, and mortality of the target insect. In the present study, different isolates were evaluated for their potential to control the whitefly *Bemisia tabaci* to assess the effect of storage on the shelf-life and efficacy of entomopathogenic fungi. The Tween 80 spore suspensions were kept under two different temperature regimes, either at 4 °C or at 26 °C; samples were taken for determination of the conidial viability and pathogenicity immediately and 1, 2, 3, 4 month following preparation. Spores viability and pathogenicity decreased significantly with the time at both temperatures inter-isolate and intra-isolate, whereas the mortality kept above 60 % for all isolates and two temperatures, but with difference in values of LD_{50}. Our results demonstrate the importance of entomopathogenic fungi as an agent microbial control with respect to the production conditions.

Contributed paper. Wednesday, 11:30. **131 STU**

Effects of production media and fertilisers on persistence and virulence of *Beauveria bassiana* F418 strain and a transformant *gfp* F418 tr1 in soil

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The clover root weevil (CRW) (*Sitona lepidus* Gyllenhal (Coleoptera: Curculionidae), is a major pest of white clover in New Zealand pastures. The entomopathogenic fungus *Beauveria bassiana* F418 strain showed good efficacy against CRW in the laboratory but control and establishment was variable in field trials.To devise better use practices for this fungal biopesticide, a better understanding of the ecology of the pathogen in soil is needed. This research aims to investigate effects of the principal abiotic, biotic and pre application factors on *B. bassiana* F418 dynamics in soil and its ability to infect insects. Clonal populations of *B. bassiana* F418 strain and a transformant (*gfp* F418 tr1) containing the green fluorescent protein gene and the hygromycin B resistance gene have been used in sterile and non sterile soils to investigate specific effects of variable factors on persistence and efficacy. Effects of production media (pre application factor) and fertilisers (abiotic factor) on fungal survival and insect infection were evaluated. The production media tested were one-tenth strength Sabouraud dextrose agar (1/10 SDA), 1/10 SDA+1% chitin, SDA and SDA+1% chitin. The soil was supplemented with a nitrogen fertiliser, a phosphate fertiliser, both or neither of them. The results of this study will be presented and discussed.

Contributed paper. Wednesday, 11:45. **132**

Effect of preying on *Metarhizium anisopliae* - infected onion thrips larvae on some behavioural parameters of *Orius albidipennis*

Hamid-Reza Pourian[1]; Reza Talaei-Hassanloui[1]; Reyhaneh Ezzati-Tabrizi[1]; Aziz Kharazi-Pakdel[1]
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The general predator *Orius albidipennis* and entomopathogenic fungus *Metarhizium anisopliae* arethe most important natural enemies of *Thrips tabaci* in most area. In this study, we estimated some behavioral parameters such as Searching Time (ST), Feeding Time (FT) and Predation Rate (PDR) in *O. albidipennis* eating on healthy and infected thrips larvae which had been infected with three concentrations of *M. anisopliae* EUT118 at four time intervals; 0, 24, 48 and 72 hours after infestation. Applied concentrations were 1×10^3, 2×10^4 and 2×10^5 conidiaml^{-1} as nearly LC_{25}, LC_{50} and LC_{75} ones for second instar-larvae of thrips, respectively. Data analysis showed that ST of predator was increased in comparison with those preying on healthy larvae and parameters PDR and FT were decreased. Fungal isolate *M. anisopliae* EUT118 could affect on above-mentioned predator parameters, it simply means that *O. albidipennis* have responded to attendance of fungus and to the infected patches by increasing the ST and decreasing FT and PDR levels. These characters of predator confront of fungus are so important in dual use of these two thrips biocontrol agents. Possible reasons for increasing or decreasing these behavioural parameters are discussed.

Contributed paper. Wednesday, 12:00. **133**

Assessing potential effects of the *Beauveria brongniartii* biological control agent on fungal community structures in soil microcosms

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Investigation of non-target effects is an important aspect for risk assessment of biological control agents (BCA). In the past investigations have focused on macroorganisms while soil microorganisms have been neglected, mainly due to the lack of suitable methods. The fungal BCA *Beauveria brongniartii*, which is commercially applied to control the European cockchafer, *Melolontha melolontha* was used to assess effects on soil fungal communities. The experimental system consisted of six soil microcosm treatments with and without *M. melolontha* larvae and included BCA- and carbofuran-based chemical control agent (CCA) treatments. Quantitative real-time PCR analysis of a specific microsatellite marker was used to quantify *B. brongniartii* in soil and fungal ribosomal intergenic spacer analysis (RISA) was applied to assess changes in fungal communities. Strongest and most significant changes in soil fungal communities were detected for treatments containing larvae that had died from either control agent. The BCA alone revealed much smaller and transient effects, while CCA effects were also small but significantly increased at the end of the experiment. The results revealed that either control strategy induced relatively small effects on soil fungal communities and that molecular genetic tools may be efficiently applied for monitoring and effect assessment of fungal BCAs.

CONTRIBUTED PAPERS Wednesday, 10:30-12:30

VIRUSES 4

Contributed paper. Wednesday, 10:30. **134**

Characterizing the physical state of covert or persistent baculovirus genomes in insect hosts

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Baculoviruses produce both overt and covert infections in lepidopteran hosts. In the covert or persistent infections, which are vertically transmitted between generations it is unclear if the virus DNA exists in an integrated state within the host genome, as an episome or within virus particles. Our lack of understanding of the physical status of covert baculovirus genomes stems partly from their very low level within the insect. Although they can be detected using polymerase chain reaction (PCR) and quantitative (Q) PCR they are often at the limits of reliable detection. Persistent baculovirus infections in laboratory insects pose serious problems since they may reactivate spontaneously or be triggered by a heterologous baculovirus infection. To characterize persistent baculovirus genomes further we have isolated total DNA from *M. brassicae*, *T. ni* and *S. exigua* insects and fractionated it using sucrose velocity gradients. Virus DNA either co-migrated or migrated apart from cellular DNA in an insect species-dependent manner. To characterize the virus DNA from sucrose gradient fractions further we amplified it in vitro using random primers with DNA polymerase. This amplified DNA could then be readily analysed using PCR to produce products that were sequenced to confirm the identity of the viruses. DIG-labelled probes were also derived from the amplified DNA. These were used to probe filters containing restriction enzyme digests of bacmids to determine if complete genome sequences were present in the original virus DNA isolated from persistent infections. These results suggested that there may be differences between the genomes of persistent viruses and their wild type counterparts.

Contributed paper. Wednesday, 10:45. **135**

Virus reactivation in *Spodoptera exigua* laboratory culture

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Baculoviruses are recognised for causing overt infections that result in host mortality. Sublethal infections may lead individuals to transmit a covert (persistent) infection vertically. Crucially, the persistent virus may spontaneously reactivate to a cytopathic form causing liquefaction of the host. Spontaneous overt infections resulting from such activation in a population suggests that the persistent virus or viral DNA remains replication competent. Reactivation of persistent viruses has been observed very occasionally in laboratory cultures of *Spodoptera exigua* over an extended period. These spontaneous infections occurred in the late larval stages that had not been exposed to any baculovirus. Restriction fragment length polymorphism analysis revealed that the reactivated virus from individual insects closely resembled either SeMNPV or *Mamestra brassicae* (Mb) NPV. Using SYBR Green based Q-PCR and virus-specific primers we have been able to successfully quantify both NPV species from covertly and overtly infected individuals. In some individuals both viral species were present. An array of restriction enzymes was used to digest overt viral DNA, which was then analysed using southern-blot hybridization with DIG-labeled probes generated from reactivated

virus DNA, persistent virus DNA, cell culture derived DNA and from SeMNPV DNA. The evidence generated supports the hypothesis that in our laboratory cultures of *S. exigua*, one or more persistent baculovirus infections are being maintained

Contributed paper. Wednesday, 11:00. **136**

Vertical transmission and persistent infection of NPVs in Eastern Spruce Budworm

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Persistent baculovirus infections have been identified in a number of host species and are proposed as a mechanism for vertical transmission of the virus. Persistent infections have been identified in a stock of *Choristoneura fumiferana* by nested PCR. Two distinct isolates have been detected, CfMNPV and CfDEFNPV. The latter is not infectious to *C. fumiferana per os* but is involved in a symbiotic relationship with CfMNPV. Both isolates appear capable of persistently infecting the host. We have attempted to establish a persistent baculovirus infection with a genetically modified CfMNPV expressing GFP, in order to differentiate vertical transmission of the inoculum virus from the pre-existing persistent infections. The potential for vertical transmission of recombinant baculoviruses has important implications for their use as biopesticides and must be considered when assessing their environmental impact. L5 *C. fumiferana* larvae were inoculated with an LD_{80} of recombinant CfMNPV, survivors were reared individually and collected and weighed as pupae. Emerging adults were mated in pairs and eggs collected from each pair daily. RNA and DNA extractions were carried out from surviving adults and offspring larvae to determine whether a persistent infection was present. GFP expression was observed in eggs resulting from pairs where one or more parent had survived virus challenge but not from unchallenged parents.

Contributed paper. Wednesday, 11:15. **137**

Within-host dynamics and virulence of co-inoculated baculovirus isolates: Evidence of synergism and antagonism

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CfMNPV and CfDEFNPV are two distinct species of baculovirus isolated from *Choristoneura fumiferana* (Eastern Spruce Budworm). CfDEFNPV has an atypical pathogenicity to the host, only being infectious *per os* in the presence of CfMNPV; this symbiotic relationship shows evidence of synergism. Bioassays presented herein suggest an altogether more subtle and complex relationship. At low-dose combinations there is evidence of synergism, but at high-dose combinations there is actually antagonism. Real-time PCR assays were developed to quantify the viral load of isolates at mortality and also to monitor within host dynamics. Daily sampling of larvae inoculated with CfMNPV showed total viral copy number increased dramatically between days 6 and 8 post inoculation before reaching a plateau. This increase occurred earlier and more rapidly in co-inoculations with CfDEFNPV. CfDEFNPV-specific DNA sequences were found to peak between day 6 and 8 and then to decline sharply, suggesting CfDEFNPV may play a more important role in early virus deaths.

Contributed paper. Wednesday, 11:30. **138 STU**

Aggregation and infection risk in Lepidoptera

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Horizontal transmission of baculovirus disease depends upon contact of a susceptible host with infectious viral particles. Theory suggests that there should be increased potential for horizontal transmission of pathogen at high host density due to the increased likelihood of contact. Gregarious species will experience high densities locally, even though host density measured regionally may be low. However, the relative importance of local versus regional population densities of lepidopteran hosts has received less attention in baculovirus research than have the impacts of overall population density. To assess the effect of spatial distribution on the transmission of viral disease within a population a number of manipulation experiments were carried out. Aggregatory configuration of either host or diet varied between treatments, whilst within-plot density was kept constant. Our host species which all feed on cabbage were *Pieris brassicae* (gregarious at the larval stage), *Mamestra brassicae* (solitary at the larval stage) and *Autographa gamma*.

Contributed paper. Wednesday, 11:45. **139 STU**

Resistance to the CpGV: Improved efficiency by selection pressure on resistant hosts

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It has been suggested that baculoviruses and their insect hosts are subject to ancient adaptation with a specialization of the pathogen. These host-pathogen interactions occur on the field and selection of the most adapted population is continuous. The recent development of resistance in the codling moth, *Cydia pomonella* against the CpGV "Mexican" strain used for its control in the orchards provides a way to test the global capacity of the virus to adapt to new host situation under selective pressure. A laboratory colony (RGV), originated from a French codling moth population, was selected for resistance to the CpGV-M. A CpGV isolate (NPP-R1) that can partially overcome the resistance was identified. NPP-R1 appears to be composed by a mixture of genotypes. In order to try to improve the virus efficacy under selective pressure, successive replication cycles were carried out on RGV. The activity of the fourth generation isolate (2016-r4) was analysed on both susceptible and resistant hosts. A significant increase of efficiency on the RGV colony was observed, with a 40 % reduction of the LC_{50} and a 7-fold decrease of the LC_{90}. However, this increase of efficiency on resistant larvae was concomitant with a slight reduction of efficiency on susceptible larvae.

Contributed paper. Wednesday, 12:00. **140**

Real-time PCR analysis of a mixed infection of granulovirus and nucleopolyhedrovirus from *Adoxophyes orana*

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A mixture of *Adoxophyes orana* granulovirus (AdorGV) and *A. orana* nucleopolyhedrovirus (AdorNPV) was recovered from *A. orana* larvae in the UK. The viruses have previously been separated, sequenced and biologically characterised. AdorGV is slow-killing with an ST_{50} (using an LD_{80} dose) of 37.0 days in neonates. AdorNPV is fast-killing with an ST_{50} (using an LD_{80} dose) of 8.8 days in neonates. As the viruses were originally found together, bioassays were performed to investigate speed of kill during a mixed infection. Neonate larvae were infected with either an LD_{80} dose of AdorGV, an LD_{80} dose of AdorNPV, and 50:50, 25:75 and 75:25 mixes of LD_{80} doses of AdorGV:AdorNPV. Larvae were observed daily for death and cadavers collected separately. Real-time PCR primers were designed to unique areas of the genomes and standard curves generated for AdorGV and AdorNPV. Real-time PCR was performed on the 217 resulting cadavers to determine the amount of GV and NPV DNA in each larva. The results showed that the GV remained at a low level and did not affect speed of kill in the 25:75 and 50:50 GV:NPV mixes, but slowed down the speed of kill and replicated prolifically after 12 d p.i. in the 75:25 GV:NPV mix.

Contributed paper. Wednesday, 12:15. **141**

The physical association of genetically distinct nucleocapsids contributes to the maintenance of nucleopolyhedrovirus diversity

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Nucleopolyhedrovirus (NPV) transmission between hosts is accomplished by occlusion derived virions (ODVs) containing single (SNPVs) or multiple (MNPVs) nucleocapsids. For MNPVs, it is believed that nucleocapsids containing genetically distinct genotypes can be enveloped into a single virion prior to occlusion. To prove this, *Spodoptera frugiperda* larvae were infected with a 1:1 mixture of two *S. frugiperda* MNPV (SfMNPV) purified genotypes, SfNIC-B and SfNIC-C. The progeny virus obtained from these larvae was inoculated in new batches of insects at five different doses. Two other batches of larvae were inoculated each with a different dose of a wild-type SfMNPV isolate containing multiple genotypes. The abundance of each genotype from each insect was then estimated by semi-quantitative PCR using specific primers. At the highest doses, viral populations behave like normal mixed infections with no differences between expected and observed progeny genotypes. However, a higher number of associated genotypes than expected occurred at the lowest doses, suggesting that genetically heterogeneous ODVs were responsible for many of the primary infections. The physical association of genetically distinct nucleocapsids is the most likely explanation for these results. This trait may guarantee the transmission of NPV diversity and, hence, survival, when OBs are scarce in the environment.

STUDENT WORKSHOP Wednesday, 12:00-14:00

Spreading the word: Skills for Communicating Science and Getting it Funded

Workshop paper. Wednesday, 12:00. **142**

Delivering oral presentations

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Delivering an oral presentation through venues such as meetings of professional societies and departmental seminars is one of the most important means of communicating your results to the scientific community. As important as oral presentations are, many scientists, even those advanced in their careers, fail to communicate effectively because they ignore common features that characterize an effective presentation. These include (1) use of a topic outline of the beginning of a presentation, (2) a short statement of the purpose of the study, often with a one sentence summary of the key results, (3) effective use of audio visual aids to present the methods and results, which today almost invariably means a PowerPoint presentation that limits the number of slides to no more than one per minute, (4) tables that in general should be no more than eleven lines in length, (5) avoidance of jargon to maintain the clarity of the presentation for audiences that often contain non-specialists, (6) a summary of the findings, generally limited to three or four key points or conclusions, and (7) acknowledgements. These principles apply to short presentations such as contributed papers, as well as to invited symposium talks and departmental seminars.

Workshop paper. Wednesday, 12:30. **143**

Editing and reviewing scientific manuscripts

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From the time you upload your manuscript to a journal website, it is in the hands of the editorial staff. Many journals have chief editors to assign each manuscript to an associate. This associate editor has the job of inviting appropriate reviewers and making decisions at steps along the way. Your paper will be read, at least briefly, by the chief editor and the associate editor before reviewers are invited. A copy of the paper will be sent to those who agree to review it. Reviewers are asked to complete their reviews, typically, within 3 to 4 weeks. Journals and editors work to minimize the amount of time it takes to reach any decision, either interim or final. If editors and reviewers are reasonably prompt, a decision may be reached within just a few weeks. Reasons for delay in the process are several, but most often are due to delays by reviewers or authors. Journals vary in their overall acceptance rate, but this can exceed 50%. In my presentation, I will describe the editing and reviewing process and offer suggestions helpful to authors.

Workshop paper. Wednesday, 13:00. **144**

Strategies for writing successful grant applications

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Unless you are independently wealthy or have a patron sponsor one of the most demanding and frustrating, though essential challenges of all scientific careers, is financing your science. This entails writing convincing grant applications to a variety of funding sources.

While there are many common factors to writing successful grant applications, there are some specific to only certain funding agencies and sometimes strategies have to change as the funding climate changes. Of these there are many "common sense" strategies, though few of us follow them. This seminar is from the perspective of someone who has been successful with grant applications, and not, and who served on the "dark side" evaluating the grant applications of others. It is a competition. There never is enough money to go around and so your application has to be better than the rest. But there are ways for you to submit the best application you can. Some simple tips, which too few people follow: Be aware of the variety of funding sources and choose ones that best reflect what you want to do research on. Start way ahead, preferably a year or at least six months to start the writing. Follow the instructions. Ask a colleague to review your grant for the science and another for content, flow, clarity, grammar and style. Do not assume that a good CV will substitute for a poorly written grant proposal. These and other "tips" will hopefully give you a head start as you begin your own scientific careers.

Workshop paper. Wednesday, 13:30. **145**

What funding agencies want: Tips for getting your research funded

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Both new and experienced investigators have to deal with the writing of grants to support their research programs. This is not a trivial task, even for veteran scientists this is always a challenge. It is a competition after all! Grant writing is perhaps not a top priority skill that most graduate students consider for their degree requirements. In most cases and even before graduate, grant-writing becomes a necessary reality that one cannot evade. Moreover, with current success rates falling to 50% or below, the difference between success and failure often results not just from the quality of the science, but from the quality of the grant application. In this presentation I will summarize major aspects to consider when writing peer-reviewed research grant applications that may apply to most granting agencies.

SYMPOSIUM (Cross-Divisional) Wednesday, 14:00-16:00

Pathogens of Bees

Symposium. Wednesday, 14:00. **146**

New insights into AFB pathogenesis

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American foulbrood (AFB) is a bacterial disease affecting the brood of the European honeybee (*Apis mellifera*). The causative agent of AFB is the gram-positive bacterium *Paenibacillus larvae* that forms extremely resilient spores, serving as the transmission stage of the bacterium. We used fluorescence *in situ*-hybridization (FISH) performed with a *P. larvae*-specific, 16S rRNA-targeted oligonucleotide probe to analyze the early steps in the pathogenesis of American foulbrood. The following chain of events could be demonstrated: (i) The spores germinate in the midgut lumen, (ii) the vegetative bacteria massively proliferate within the midgut before (iii) they start to locally breach the epithelium and invade the haemocoel. Our results implicated that successful colonization of the gut may be one of the key factors in AFB pathogenesis. The paracellular route was shown to be the main mechanism for invasion contrasting earlier hypotheses of phagocytosis of *Paenibacillus larvae*. Invasion coincided with the death of the host implicating that the penetration of the midgut epithelium is a critical step determining the time of death.

Symposium. Wednesday, 14:24. **147**

***Nosema* in bumble bees: Steps towards understanding**

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The relationship between *Nosema bombi* and its bumble bee hosts has long been controversial. While its route of infection within hosts is well-known, transmission routes among hosts have remained unclarified. Furthermore, the impact of the parasite on host fitness (its virulence or pathogenicity) has been reported as high, non-existent or even, counter-intuitively, beneficial. Most of this confusion is due to a lack of controlled experiments. Recent work in a number of groups has considerably advanced our knowledge. In this talk I will review our current understanding and give directions for future work on this important host-parasite relationship

Symposium. Wednesday, 14:48. **148**

Sexual transmission of deformed wing virus in honeybees

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Deformed wing virus (DWV) infected semen was used for artificial insemination of DWV-free virgin queens. High titres of DWV could subsequently be detected not only in the spermatheca, but also in the ovaries, demonstrating venereal transmission of DWV in honey bees. Subsequent vertical transmission of the virus to the progeny of DWV infected queens was also demonstrated. Neither transmission route is 100% effective. Whether venereal transmission of DWV occurs during natural mating remains to be determined. The implications for the use, sale and transport of semen samples for artificial insemination are discussed.

Symposium. Wednesday, 15:12. **149**

Epizootiological aspects of chalkbrood infections in the alfalfa leafcutting bee

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Chalkbrood is a disease of bee larvae caused by fungi in the genus Ascosphaera (Ascomycetes: Ascosphaerales). These fungi have been found only in association with bees, either as pathogens or saprophytes on pollen that is stored in bee nests. Like most entomopathogenic fungi, spores are the infective stage, but these spores differ in that they are unable to infect through the host cuticle; they must be consumed by the larvae and infect through the gut. Thus, the epizootiology of chalkbrood is affected by the ability of the fungus to transmit spores to the host' pollen food stores. This is most likely achieved by phoresy on the adult bees. In addition, epizootics are uncommon in honey bees, but can be very common in managed solitary bees such as the alfalfa leafcutting bee and the blue orchard bee. This difference is probably a result of the capacity of honey bees to control hive temperatures above that which is optimal for disease development, and to remove diseased larvae from the nest. But it may also be due to the nest construction differences. Newly emerging adult cavity nesting bees may have greater exposure to the spores than do adult honey bees.

Symposium. Wednesday, 15:36. **150**

Co-evolution of mites and social honeybees in Asia

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Most species of Asian honeybees host a parasitic mite and some of these mites have switched-host to the Western honeybee (*Apis mellifera*) to become serious pests. Here I look at evidence of co-evolution in two Asian honeybee/mite host-parasite systems; (a) Eastern honeybees/*Varroa* mites and (b) giant Asian honeybees/*Tropilaelaps* mites. Three species of mite within the genus *Varroa* (*V. jacobsoni*, *V. destructor* and *V. underwoodi*) are external parasites of the Eastern honeybee (*A. cerana*) throughout Asia, and a fourth species (*V. rindereri*) parasitises the red honeybee of Borneo (*A. koschevnikovi*). Evidence from morphological, behavioural and biogeographical studies implies that these mites and bees have co-evolved. The degree of association between external parasitic *Tropilaelaps* mites and giant honeybees has long been questioned because (a) the first species of *Tropilaelaps* discovered (*T. clareae*) was initially isolated in the Philippines from introduced Western honeybees and from field rats that were nesting nearby, (b) *Tropilaelaps* mites cannot feed on adult honeybees and can only survive in the absence of honeybee brood for about one week, yet giant honeybees have migratory habits and often exit for long periods without brood and, (c) the chelicerae of *Tropilaelaps* mites are morphologically more similar to those of predatory rather than parasitic mites. Nevertheless, because *Tropilaelaps* mites have been found infesting giant honeybees throughout Asia those bees are now widely regarded as the mites' primary host. Recent evidence from patterns of *Tropilaelaps* and giant honeybee speciation and from biogeographical studies confirms that giant honeybees are the primary host of these mites and implies that the bees and mites have co-evolved. In this talk I review published evidence of co-evolution in these two host-parasite systems. I also report some preliminary findings of evidence of co-evolution in both systems by analysing mite and bee phylogenies in the evolution model 'Tarzan', an event-based tool that assigns costs to evolutionary events such as co-speciation, duplication, sorting and switching events.

CONTRIBUTED PAPERS Wednesday, 14:00-16:00

BACTERIA 3

Contributed paper. Wednesday, 14:00. **151**

Specificity of *Bacillus thuringiensis* delta-endotoxins: A review, finally…

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Many data on insecticidal activity of delta-endotoxins from *B. thuringiensis* have been published since *cry* genes were first cloned. The Bt toxin specificity database[1], now containing 1782 bioassays involving 793 toxin preparations and 164 species, is facilitating the first review of those data. Bioassays of Cry1, Cry2 and Cry9 toxins account for 96% of all assays conducted against Lepidoptera. The most frequently tested family is the Noctuidae (44% of total assays), followed by Pyralidae (12.2%), Plutellidae (9.8%) and Tortricidae (8.8%). Permissiveness at the family level (proportion of positive species-toxin combinations) was lowest in the Noctuidae (40%) and highest in the Pyralidae and Tortricidae (90 and 94%). The proportion of active toxin types ranged from 18% for the most recalcitrant species (*Agrotis ipsilon*) to 80% for the least (*Ostrinia nubilalis*). Cry1A and 2A toxins had the broadest activity spectrum, with >85% of the species tested being responsive. Comparing toxicity values for a subset of toxin-species combinations (surface layering assays, neonate larvae, >20 assays) revealed that the lowest LD_{50}s are in the 0.5 to 1.5 ng toxin per cm^2 range (Cry1A toxins against a variety of species). Similar analyses are in progress for assays conducted with Coleoptera and Diptera. [1] (http://www.glfc.forestry.ca/bacillus)

Contributed paper. Wednesday, 14:15. **152**

Gut flora not required for pathogenicity in *Bacillus thuringiensis* infecting diamondback moth

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Recent experimental work has indicated that Bacillus thuringiensis (Bt) cannot exploit aseptic hosts experimentally cured of their gut flora. These findings remain controversial because previous reports suggested that the gut flora competes with B. thuringiensis in the host. Two explanations for this discrepancy include: (1) Bt biopesticide strains are attenuated and therefore not able to grow in aseptic insects or (2) Bt is sensitive to the antibiotic treatments used to make insects aseptic. We developed methods for producing aseptic diamondback moth that did not require treating larvae with antibiotics. We tested the effect of exposing larvae to Enterobacter and/or antibiotics (rifampicin) on the mortality caused by standard and rifampicin resistant (rifR) Bt A second experiment tested whether diverse strains (wild types; biopesticide isolates and passaged rifR strains) could grow in rifampicin treated larvae or in untreated aseptic larvae. Rifampicin treated insects only reduced larval mortality for rifampicin sensitive Bt but not rifR Bt. All strains could grow in untreated aseptic larvae. The addition of Enterobacter to larval diet slightly reduced the mortality rate of an HD1 strain suggesting that the gut flora may have some protective role for the host, as found in other systems.

Contributed paper. Wednesday, 14:30. **153**

Pathogenesis of *Bacillus thuringiensis* subsp. *kurstaki* in spruce budworm and gypsy moth

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Midgut microflora can play a significant role in pathogenesis of *Bacillus thuringiensis* (Bt), depending on host and bacterial (sub)species. Crystal protein damage to the midgut can facilitate lethal septicemia by intestinal bacteria, followed by proliferation of Bt in the cadavers. In some hosts, Bts have no ability to proliferate independently of midgut microbiota, whereas in others the pathogen grows much better in their absence. We used dilution plating and microscopy to examine the changes in abundance of Bt and midgut bacteria following ingestion of a lethal dose of HD-1. In both species, there is a rapid drop in number of Bt colony forming units (cfu) over 32 h post ingestion (hpi). In spruce budworm, Bt multiplication commences 48 hpi in dead or moribund larvae, reaching densities of $>10^{10}$ cfu per larva at 72-96 hpi. In gypsy moth, there is no evidence of massive Bt multiplication, with maximum cell densities reaching at best pre-ingestion levels ($\sim10^5$cfu/larva) at 72-96 hpi. In Bt-infected spruce budworm larvae there was little change in abundance of *Streptococcus* and *Staphylococcus* relative to untreated larvae, whereas both species increased dramatically in treated gypsy moth larvae. Further experiments are in progress.

Contributed paper. Wednesday, 14:45. **154 STU**

Distinct changes in immune system are associated with *Bt* exposure in *Bt*-resistant and *Bt*- susceptible *Trichoplusia ni* colonies.

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Increasing evidence implicates a role for the innate immune system of *Trichoplusia ni* in mitigating *Bacillus thuringiensis kurstaki* (*Btk*) toxicity. We studied the immune response to *Btk* in susceptible and resistant *T. ni* by measuring the resistance levels, the expression of hemolymph antimicrobial proteins (AMP), and the differential number of circulating hemocytes in individual larvae. The immune response was evaluated after injections of a cocktail of gram negative (*Escherichia coli*) and gram positive (*Staphylococcus epidermidis*) bacteria, and after *per os* exposure to a commercial formulation of *Btk* endotoxins. The differential expression of genes encoding several AMPs were assessed in fat body and midgut tissues by quantitative real-time PCR 8, 24, and 48 hours after treatments. The protein-level changes in the hemolymph were determined by reverse-phase HPLC analysis, and antimicrobial activity assays. Exposure to treatments caused significant increases in the number of circulating hemocytes, the levels of AMP gene expression, as well as changes in hemolymph protein composition. The overall increase in cellular and humoral immune factors after exposure to *Btk* suggests that multiple systems are stimulated by exposure, and may contribute to reducing the toxicity of *Btk*.

Contributed paper. Wednesday, 15:00. **155 STU**

Characterization of intracellular response in mosquitoes to *Bacillus thuringiensis* Cry11Aa toxin

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Dengue and malaria are human diseases produced by arbovirus and *Plasmodium*, respectively. Control measures against these diseases have focused on mosquitoes because both pathogens require dipteran to infect their hosts. *Bacillus thuringiensis* (*Bt*) subspecies *israelensis (Bti)* produces protein crystals (Cry) that are highly toxic to larvae of *Aedes* and *Anopheles* mosquitoes. Cry proteins show a sequential binding mechanism to specific receptors. One of these receptors is anchored into lipid rafts. It has been proposed that a high concentration of the toxin in the lipid rafts has the dual effect of induction of the formation of pores and subsequent death and/or the activation of intracellular signaling pathways. Recently it has been suggested that invertebrates are able to acquire resistance against *Bt* through defense responses. The p38 mitogen activated protein kinase route of cellular signaling was linked with a defense response against Bt toxins in *Caenorhabditis elegans*. Gene silencing of p38 has been previously shown to increase the specific suceptibility of *C elegans* to Bt toxins. In this work multiple strategies were used to study the participation of p38 in mosquito resistance to Bti Cry11Aa toxin and to explore other proteins implicated in this process.

Contributed paper. Wednesday, 15:15. **156 STU**

Kinetics of microbial degradation and chemical fixation of Cry 1Aa Bt toxin in various soils

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Genetically modified crops, which produce Cry pesticidal proteins from *Bacillus thuringiensis*, release the toxins into soils through root exudates and upon decomposition of residues. Although, gene transfer and resistance emergence phenomena are well documented, the fate of these toxins in soil has not yet been clearly elucidated. Cry proteins, in common with other proteins are adsorbed on soils and soil components. The orientation of the molecule and conformational changes on surfaces may modify the toxicity and confer some protection against microbial degradation. Current detection methods require the toxin to be chemically extracted from soil. It is difficult to distinguish between degradation and decreasing efficiency of chemical extraction prior to detection due to chemical fixation. The aim of this study is to follow the fate of Cry 1Aa added to contrasting soils subjected to different treatments to inhibit biological activity.

Contributed paper. Wednesday, 15:30. **157 STU**

The *ger* genes of pBtoxis are responsible for the alkaline-activation of germination in *Bacillus thuringiensis* subsp. *israelensis*.

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Bacillus thuringiensis subsp. *israelensis* (Bti) encodes multiple spore-associated toxins on the 128kb plasmid pBtoxis. Numerous other proteins are encoded by this plasmid but their influence on the host bacterium is unknown. Amongst the pBtoxis genes are three putative germination (*ger*) genes that appear to be organised into a single operon. Comparison of the germination responses of spores from Bti strains with and without pBtoxis revealed that pBtoxis can promote the germination of the host. However, no change in response is observed when spores are activated by heat treatment: enhancement of germination is only seen following alkaline activation of the spores. Introduction of the *ger* operon on a recombinant plasmid to the plasmidless Bti strain establishes its role in this response. This is the first identification of *ger* genes that are responsible for alkaline activation. There is an obvious physiological relevance to such a response in bacteria that, during their pathogenesis, damage the mosquito larval gut and must germinate in this alkaline environment to exploit the insect cadaver as a source of nutrients for growth and colonisation. Co-transmission of these ger genes with the genes encoding insecticidal toxins may be beneficial to the host bacilli.

Contributed paper. Wednesday, 15:45. **158 STU**

Laboratory-selected Cry1Ac-resistant *Helicoverpa zea* (Lepidoptera: Noctuidae) cannot survive on *Bt* cotton: Implication of potential synergistic interactions of Cry1Ac and gossypol

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Laboratory experiments with field-cultivated *Bacillus thuringiensis* (*Bt*) and non *Bt* cotton squares were conducted with Cry1Ac-resistant (AR) and susceptible (SC) *Helicoverpa zea* (Boddie). More than 150-fold resistant AR had significantly higher larval survivorship after feeding on *Bt* cotton squares compared to SC. AR significantly outperformed SC in numbers of survivors, highest number of larval instar reached, and duration of larval survival. However, AR could not complete larval development on *Bt* cotton. Additionally, a significantly lower percentage of AR (25%) larvae reached pupation on NBt compared with SC (31%). Before additional experiments were conducted, AR was crossed with a new susceptible lab colony (SC1) to increase fitness (AR1). Diet incorporation bioassays were conducted with Cry1Ac (15 µg/g) and gossypol (0.15%) and their 2, 4 & 8-fold dilutions to help determine the contribution of these compounds at concentrations observed in *Bt* and NBt cotton. Cry1Ac at 15 µg/g was significantly more lethal to SC1 compared to AR1; however, no differential susceptibility was observed in strains for 0.15% gossypol. Combinations of Cry1Ac and gossypol were synergistic against AR1, but not to SC1. These results may help understand the inability of AR to complete development on *Bt* cotton, and therefore may help explain the absence of field-evolved resistance to *Bt* cotton by *H. zea*.

CONTRIBUTED PAPERS Wednesday, 14:00-15:30

NEMATODES 3

Contributed paper. Wednesday, 14:00. **159**

Are there differences in dispersal, infectivity and sex ratio between early or late emerging infective juveniles of *Steinernema carpocapsae?*

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Several studies have demonstrated biological differences of infective juveniles (IJs) of entomopathogenic nematodes emerging directly from a cadaver into soil compared with IJs from a cadaver emerging into water, held in water, and then applied to soil. We further elucidated differences between *Steinernema carpocapsae* IJs that emerged directly from a cadaver vs. those that emerged from a cadaver and were held in water. Our objective was to compare dispersed and non-dispersed IJs from a cadaver vs. those held in water between two time periods designated as early (first 2 days) or late emerging IJs (7th day). Our data showed that (1) a significantly higher proportion of early emerging IJs from the cadaver treatment dispersed compared with late emerging IJs from a cadaver or either group of emerging IJs held in aqueous suspension, (2) IJs from cadavers were more infectious than those from the aqueous suspensions and IJs that dispersed were less infectious than those that did not disperse, and (3) IJs that emerged early were mostly males whereas those that emerged late were mostly females. For the non-dispersed IJs, most of them that emerged early were males and those that emerged later were females but among dispersing IJs, there was no difference in sex ratio between early and late emerging nematodes.

Contributed paper. Wednesday, 14:15. **160**

Male *Steinernema longicaudum* do not sexually mature in the absence of female

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We demonstrate that solitary-reared males of *Steinernema longicaudum* Shen & Wang (Nematoda: Steinernematidae) remain sexually immature until exposed to a female. Only following exposure to a female for a sufficient period of time males become fertilisation competent, with the seminal vesicle containing sperm. Experiments carried out in insect blood showed that most males require at least 18 hours with a female before they contain sperm. In contrast, most solitary-reared females were ready to mate and were fertilised by an already mated male within one hour of pairing. Males separated from females by a permeable barrier produced as many sperm as males that were in physical contact with females, indicating that male maturation is mediated by chemical cues released by females. The need of a male for female presence before he becomes fertilisation competent is unknown in animals. This unusual requirement of a male *S. longicaudum* for a female to be present before he produces sperm may be related to the habitat of the reproductive populations within dead insects. We suggest that arrested male maturation is an adaptive strategy that reserves the male's resource for investment in protracted survival while he awaits the arrival of a potential mate.

Contributed paper. Wednesday, 14:30. **161 STU**

Habitat preferences of nictating nematodes

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The nictation behaviour of entompathogenic nematodes; *Steinernmea* carpocapsae, *S. feltiae*, *S. glaseri*, *S.* scapterisci, *Heterorhabditis* megidis, and *H. bacteriophora* was observed over a 10 day period. Infective juveniles (IJs) were given a choice of four habitats (sand, peat, soil, and leaf litter) in the presence and absence of a host insect, *Galleria* mellonella. The number of nictating nematodes on each habitat was recorded. The slug parasitic nematode, *Phasmarhabditis hermaphrodita* and *Caenorhabditis elegans* was also included in our analysis. Our observations indicate that these species exhibit different habitat preferences playing a key role in their behaviour

Contributed paper. Wednesday, 14:45. **162 STU**

Variability in desiccation tolerance among different strains of the entomopathogenic nematode *Heterorhabditis bacteriophora* Poinar

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The entomopathogenic nematode *Heterorhabditis bacteriophora* Poinar is used for biological control of several soil-borne insect pests. As compared to steinernematid nematodes, the shelf life of *H. bacteriophora* is rather shorter and nematodes loose infectivity faster. In order to increase shelf life, the metabolism of these nematodes during storage must be minimised by means of desiccation of dauer juveniles (DJs). Previous investigations indicate

that the heritability of the desiccation tolerance is high provided DJs have been adapted to moderate desiccation conditions. This makes this trait an excellent target for genetic selection. Positive results in enhancement of desiccation tolerance have already been obtained. In order to start selection with a broader genetic background, this investigation screened the desiccation tolerance of sixty-one *H. bacteriophora* strains from different geo-climatic regions. Dehydrating conditions were produced by treating DJs with non-ionic polymer polyethyleneglycol 600 solution (PEG 600). Desiccation was measured as water activity (a_w-values) obtained from PEG 600. The *H. bacteriophora* strains were produced *in vivo* using the greater wax moth, *Galleria mellonella* (Lepidoptera, Pyralidae). All treatments were done with one nematode batch and repeated three times. Significant intra-specific variations ($\alpha \leq 0,05$) were noted among *H. bacteriophora* strains. Mean desiccation tolerance ranged from a_w-value 0,90 to of 0,95 for non-adapted nematode populations and 0.76 to 0.99 for adapted nematode populations. Variability within one *H. bacteriophora* population increased with increasing desiccation stress. Strains from arid regions tolerated desiccation better than those from temperate regions. Results indicated nematode strains from Israel (a_w-value of 0,845), Germany (a_w-value of 0,857) and Egypt (a_w-value of 0,86) were the most tolerant and will be crossed for production of the foundation strain.

Contributed paper. Wednesday, 15:00. **163 STU**

Analysis of the population development of *S. carpocapsae* and *S. feltiae* in liquid culture

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Mass production of *Steinernema carpocapsae* and *S. feltiae* is carried out in monoxenic liquid culture of their symbiotic bacteria, *Xenorhabdus* spp. Parameters for successful production are the percentage of developed adults among inoculated dauer juveniles (DJs) (recovery), the DJ yield and the process time. The influence of bacteria and DJ inoculum density and process temperature was investigated. Higher bacterial density induced higher recovery, while different DJ inoculum densities had no impact. The fecundity of parental females was reduced in higher inoculum density and most of the progeny juveniles developed directly to DJs while the progeny in lower inoculum density continued to develop to another generation of adults. According to the results obtained on the relation of inoculum density and fecundity, the optimal DJ inoculum density is between 3,000 and 5,000 DJs/ml for *S. carpocapsae* and *S. feltiae*, respectively. At different process temperatures, recovery was constant. The fecundity of both species was suppressed at highest temperature at 31°C for *S. carpocapsae* and 27°C for *S. feltiae* and the DJ yield reached only half of the density recorded in cultures at lower temperatures.

Contributed paper. Wednesday, 15:15. **164**

Hunter to be hunted: Predator mites and entomopathogenic nematodes

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Sancassania sp. (Acari: Acaridae), isolated from field-collected scarab larvae, preyed on the infective juveniles (IJs) of entomopathogenic nematodes. Adult female mites consumed more than 90% of *Steinernema feltiae* IJs on an agar substrate within 24 h. When the mites were placed with *S. feltiae* IJs for 24 h and then exposed to *Galleria mellonella* (Lepidoptera: Pyralidae) larvae, the number of IJs penetrating into the larvae was significantly lower compared to IJs not exposed to mites. Mites found and consumed IJs when mites and IJs were placed in a 10-cm soil column together. The mites consumed the IJs regardless of where the mites or IJs were placed initially in the soil column. However, soil type significantly affected the predation rate of IJs by the mites with more IJs consumed in sandy soil than in loamy soil. We also observed that the mites consumed more *S. feltiae* IJs than *Heterorhabditis bacteriophora* (Rhabditida: Heterorhabditidae) IJs. No phoretic relationship was observed between predatory mites and nematodes andthe nematodes did not infect the mites.

CONTRIBUTED PAPERS Wednesday, 14:00-16:00

VIRUSES 5

Contributed paper. Wednesday, 14:00. **165**

Baculovirus IE2 forms nuclear bodies in the nucleus and enhances CMV promoter expression in mammalian cells

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In this study, we show that baculovirus immediate-early protein IE2 is a strong promiscuous trans-activator in mammalian cells. Both CMV and SV40 promoters were dramatically up-regulated by co-expression of this baculovirus protein in Vero E6 cells. This effect was most likely resulting from the ability of IE2 to compartmentalize nucleus space, and form transcription active centers. IE2 forms distinct nuclear bodies within nucleus, which were found to associate with RNA polymerase II. We also observed that both PML and SUMO-1 appeared to partially cover, or associate with the outer edges of the IE2 nuclear bodies. Mutation analysis showed that both the RING and the coil-coil domains of IE2 were essential for IE2 activation of CMV promoter in mammalian cells, while mutations at the predicted sumoylation site had no obvious effect. Treatments of siRNAs specific for PML and UBC9 (the sumoylation E2 enzyme) improved the effect of IE2 activation, suggesting that PML and other unknown sumoylated factors may be negative regulators for IE2 activation of CMV promoter. This discovery not only advanced baculovirus as a viable tool for protein expression in mammalian system, but also demonstrated the fundamental role of nuclear bodies in mammalian transcriptional control.

Contributed paper. Wednesday, 14:15. **166**

P35 is required for production of robust budded virus during AcMNPV infection of *Trichoplusia ni*

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Infection of *Trichoplusia ni* with the baculovirus *Autographa californica* M nucleopolyhedrovirus (AcMNPV) results in the melting and liquefaction of the caterpillar. However, mutants of AcMNPV that do not express the anti-apoptotic protein P35 do not liquefy the host, even though the mutant virus has LC50 and LT50 values that are similar to wild type AcMNPV. We previously reported that chitinase and cathepsin expression and activity are normal in TN-368 cells infected with the *p35* mutant, but that there is a delay in virus exit from the endosome for the mutant virus compared to wild type. In a continuation of this study, we observed that TN-368 cells infected with the *p35* deletion mutant have a low level of caspase activity, even though the cells are resistant to apoptosis. We also found that the *p35* mutant virus is less stable than wild type AcMNPV. This led us to hypothesize that the observed entry defect may be due to the virions being somehow damaged by caspases, either directly or indirectly. When the *p35* deletion virus was grown in the presence of a chemical caspase inhibitor and the entry assay was repeated, the entry phenotype was rescued. This suggests that, even in *T. ni* cells, which do not die by apoptosis in the absence of P35, P35 is still needed to inhibit caspase activity and produce robust virus particles. The lack of liquefaction with the *p35* deletion virus may therefore be due to damage to the progeny budded virus that is produced during infection, and this virus not being as efficient at spreading infection in the host. This may result in an overall weaker infection phenotype which is sufficient to kill the larvae but not to cause liquefaction.

Contributed paper. Wednesday, 14:30. **167**

AcMNPV DNA replication is essential for P47 but not for LEF-4 expression

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Baculovirus genes have been assigned to four temporal classes (immediate and delayed early, late and very late genes) depending on their timing of expression with respect to the initiation of DNA replication. The role DNA replication plays in delayed early gene expression is not clear. To investigate this, DNA replication essential genes (*lef-3* and *p143*) were knocked out via bacmid technology. Protein expression patterns in knockout transfected cells were determined using antibodies against IE-1, LEF-3, P143, DNA polymerase, LEF-4 and P47. Both *lef-3* and *p143* knockouts resulted in lower level of IE-1 expression compare with the wild type bacmid transfection. This suggested that early genes are transcribed from input viral DNA but enhanced levels of early genes do occur, most likely from progeny viral DNA template. LEF-4, one subunit of the viral RNA polymerase, was detected in the cells transfected with *lef-3* or *p143* knockout bacmids, but no P47 was detected in these cells. Protein expression in cells infected with temperature sensitive mutants defective in P47 or LEF-4 were also investigated. LEF-4 was detected in ts317 (*p47* defective) infected cells, but no P47 was detected in ts538 (*lef-4* defective) infected cells. Because LEF-4 but not P47 was detected in the absence of viral DNA replication, this suggests that only newly synthesised DNA can act as a template for p47 expression. These data indicate that progeny viral DNA plays an important role in the early to late gene expression switch.

Contributed paper. Wednesday, 14:45. **168 STU**

Characterization of AcMNPV late expression factor 3 (LEF-3) functional domains for their role in nuclear localization and baculovirus DNA replication

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AcMNPV is the best-studied member of the *Baculoviridae* family and most of the genes identified in this virus serve as a basis for comparison to other baculoviruses. A single-stranded DNA binding protein, LEF-3 (407 aa, 45 kDa), is essential for AcMNPV (Ac) DNA replication. LEF-3 also transports P143, a helicase, to the nucleus. We predict that LEF-3 has functional domains including ones responsible for ssDNA binding, P143 interaction, and nuclear localization. Site-directed mutagenesis revealed that N-terminal aa 5 to 56 are responsible for nuclear localization (NLS), while aa 2 to 125 are required for P143 interaction. Alignment of type I NPV LEF-3s revealed a region (aa 20-28 in AcLEF-3) not present in all species. Fluorescence microscopy showed that aa 3-48 of CfMNPV (Cf) LEF-3 are sufficient for nuclear import, suggesting that aa 20-28 of AcLEF-3 are not essential for the NLS. Substitution of conserved basic residues with nonpolar residues did not affect nuclear localization. However, combining individual mutations with the deletion of aa 20-28 resulted in cytoplasmic AcLEF-3. This suggests a novel system for nuclear import that may involve the structure of LEF-3. The possibility that AcLEF-3 has developed multiple NLS sequences to enhance replication efficiency is also being considered. A similar approach was used within aa 2-125 to investigate LEF- 3/P143 interaction. Preliminary results show that deleting Gly552 in P143 inhibits interaction with LEF-3. Transient replication assays show that LEF-3 function extends beyond nuclear localization and transport of P143. To further characterize the role of LEF-3 in DNA replication and late gene expression, the ability of various LEF-3 mutants to rescue function in the presence of a knockout bacmid will be reported.

Contributed paper. Wednesday, 15:00. **169 STU**

Removal of transposon target sites from AcMNPV *fp25k* delayed incidence of the FP phenotype but had no impact on DIP production in cell culture

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Low cost, large-scale production of the baculovirus *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) using continuous insect cell culture is seriously hindered by undesirable mutations in the baculovirus genome. Overcoming such mutations is an important step in enabling large-scale production of baculovirus biopesticides at a cost competitive with chemical pesticides. Few polyhedra (FP) mutants and defective interfering particles (DIP) are commonly responsible for the reduction in occluded virus yield with decreased infectivity. FP mutations commonly result from the insertion of transposons into the baculovirus *fp25k* gene. We demonstrated that removal of the transposon target sites from the wild type baculovirus *fp25k* gene (stabilized virus) reduced the incidence of the FP phenotype in late passages (passage 15), but did not eliminate the FP phenotype in very late passages (passage 30). Genotypic and phenotypic analysis of late passaged virus showed that deletion of genomic sequences also contributed to the FP phenotype and reduced infectivity. Production of DIP's with deleted sequences was shown for both WT and stabilized virus by characterization of the geometric size distribution and DNA size distribution of early and late passaged virus.

Contributed paper. Wednesday, 15:15. **170 STU**

Structural and functional analysis of the Chilo iridescent virus DNA polymerase promoter

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The delayed-early DNA polymerase (DNApol) promoter of *Chilo iridescent virus* (CIV) was dissected by deletion and site specific mutagenesis. The effects of the mutations were examined in a luciferase reporter assay using *Bombyx mori* cells transfected with promoter constructs and superinfected with CIV. An AAAAT motif located between -19 and -15 proved essential for promoter activity. Such an AAAAT motif was also found in the DNApol promoter region of other iridoviruses as well as in other putative CIV delayed-early promoters. South-Western analysis showed that a 100 kDa protein present in CIV-infected cell nuclei specifically interacted with the DNApol promoter, but not when a mutation in the AAAAT motif was made. This 100 kDa protein is considered virus specific or virus-induced because with extracts of mock-infected cells no binding was observed. Proteins with molecular masses around 100 kDa are predicted for the CIV ORFs 022L, 045L, 050L, 085L, 176R, 179R, 184R, 261R, 295R, 396L and 428L (Jakob *et al.*, 2001), and include DNA topoisomerase II and the large and a small subunit of DNA-dependent RNA polymerase. This study contributes to the further understanding of the control mechanisms of Iridoviruses transcription and the viral proteins involved.

Contributed paper. Wednesday, 15:30. **171 STU**

Suppression of AcMNPV gene expression in mammalian cells

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Mechanisms for the insusceptibility of mammalian cells to proliferative infection with entomopathogenic viruses are not well understood. The baculovirus Autographa californica multiple nucleopolyhedrovirus (AcMNPV) is used as a biopesticide and a safer viral vector in mammalian cells with potential applications in gene therapy. However, there is evidence that AcMNPV is capable of expressing viral genes at the transcriptional level at least in mammalian cells, emphasizing the importance of studying the molecular details of baculovirus-mammalian cell interaction to reinforce the safety of AcMNPV. In this study, we show that histone deacetylation acts as a suppressor for the transcription of AcMNPV in mammalian BHK cells. Real-time PCR and chromatin immunoprecipitation with a HDAC inhibitor revealed an important relationship between the viral gene expression and the histone deacetylation. On the other hand, we could not see the participation of histone methylation and HP1 binding to virus DNA in this regulation. These results provide experimental evidence that the epigenetic gene regulatory mechanism, histone acetylation at least, acts as a defense against baculoviruses in mammalian cells.

Contributed paper. Wednesday, 15:45. **172**

SV40 polyadenylation (pA) signal increases transcription but reduces protein production in baculovirus expression vector system

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Baculovirus has been widely used to produce foreign proteins in insect cells and insects. To boost protein production, the simian virus 40 polyadenylation signal or SV40 poly(A) has been used in some baculovirus expression vector systems (BEVS) . We investigated the effect of the SV40 poly(A) on the expression levels of the enhanced green fluorescent protein gene (*egfp*) in BEVS. An expression cassette with the *egfp* gene under the *polh* promoter control with and without SV40 poly(A) was inserted at the *polyhedrin*, *ecdysteroid UDP-glucosyltransferase* (*egt*) and *gp37* loci of *Autographa californica* nucleopolyhedroviruses (AcMNPV). Recombinant viruses containing the desired sequences were obtained and used to infect Sf21 cells to examine the levels of *egfp* transcription and translation. Spectrofluorometry and Western blot analyses showed that SV40 poly(A) reduced EGFP production at the three loci. However, real-time quantitative PCR and dot blot analyses showed that the *egfp* mRNA levels increased in the three viral constructs with SV40 poly(A) compared to those without SV40 poly(A). Therefore, we concluded that the SV40 poly(A) increases mRNA transcription but decreases protein production and it should be replaced with AcMNPV late gene termination sequences in BEVS.

Wednesday, 16:30-18:30

POSTERS – 2

MICROBIAL CONTROL

Poster / Microbial Control. Wednesday, 16:30. **MC-00**

Production and evaluation of mosquitocidal efficacy of *Bacillus thuringiensis* subsp. *israelensis* based formulations in Vietnam

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Bacillus thuringiensis subsp. *israelensis* strain Bti-11 based biological mosquito larvicides produced in Vietnam in different slowly released solid formulations were laboratory evaluated activity against main vector mosquitoes, *Anopheles minimus*, *Aedes aegypti*, *Culex quinquefasciatus*. The formulations were made in the small round cake form with 3 cm diameter, 2-5 mm thickness. Raw materials used were cheap and available in Vietnam: corn cob, sugar cane bagasse, cork, popcorn. Results showed that the formulation made of corn cob (CT1), the formulation made of sugar cane bagasse and polyvinylalcohol adhesive (CT4) got the highest efficacy, 96.6 and 100 % respectively. In experiment for long-term effect of products, the formulations CT_1, CT4 and CT7 had high efficiency maintenance, larvae mortality was 95% after 11 days. The formulation CT4 was remarkably degraded after 11 days, while the formulation CT1 was not noticeably degraded. The formulation CT1 was used for field trail in some ponds in urbanizing areas in Thanh Xuan district, Hanoi city. Mosquito larvae density in experimental ponds reduced from 90.8 to 100 % after 72 hours of treatment. These indicate that Bti preparations produced in Vietnam have high efficacy in the field condition and could be promising products for mosquito control programs.

Poster / Microbial Control. Wednesday, 16:30. **MC-01**

Comparison of phytopathogenic antagonism between *Bacillus subtilis* and *Bacillus thuringiensis* strains transformed with *chi*A gene from *Serratia marcescens* ATCC990

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Production of chitinase is correlated with antagonistic activities against plant pathogens in certain bacteria. *Serratia marcescens*, a Gram-negative bacterium, is one of the most efficient bacteria for degradation of chitin. The best known of the chitinolytic enzymes upon induction with chitin is the secreted chitinase (ChiA) from *S. marcescens*. In order to investigate the effect of chitinase on the antifungal activity of *Bacillus subtilis* S3, *B. subtilis* ISW1214 (competent cell) and *B. thuringiensis cry* ¯ B, the *chi*A gene of *S. marcescens* ATCC990 was transformed separately into respective bacterium. Extracellular chitinase of transformatswas grown in LB broth and detected by a chitinase assay. The recombinant enzyme was analyzed for chitinase activity and the highest activity occurred at the sixth day. A 1.5-1.7 fold activity was observed in transformants TBs.S3 and TBt.*cry* ¯ B as compared to TBs.1214. The appearance of clear zone on 1% chitin agar plate produced by transformants TBs.S3 and TBt.*cry* ¯ B was 12-15 days ahead of transformant TBs.1214. A dual-culture bioassay conducted on cultured supernatant (without cells) showed that in comparison to transformants TBs.1214 and TBt.*cry* ¯ B, TBs.S3 exhibited higher activity against 16 plant fungal pathogens tested. Transformant TBs.S3 exhibited higher antifungal activity against *Sclerotium rolfsii* and *Pythium myriotylum* than its host (*B. subtilis* S3). Transformant TBt.*cry* ¯ B exhibited higher antifungal activity against *P. myriotylum* than its host (*B. thuringiensis cry* ¯ B).

Poster / Microbial Control. Wednesday, 16:30. **MC-02**

Construction of a recombinant *Bacillus subtilis* strain as an integrated control agent being able to control to plant diseases and insect pests

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A new *Bacillus subtilis* isolate showed high anti-fungal activities (more than 80% control efficacy) against several plant diseases such as rice blast (*Magnaporthe grisea*), tomato gray mold (*Botrytis cinerea*), tomato late blight (*Phytophthora infestans*) and wheat leaf rust (*Puccinia recondita*). We tried to confer an insecticidal activity to this *B. subtilis* isolate for constructing a recombinant strain which has dual functions, anti-fungal and insecticidal activity. The insecticidal *cry1Ac* gene of *B. thuringiensis* was constructed under its own promoter in a minimal *E. coli-B. thuringiensis* shuttle vector (pHT1K-1Ac). The plasmid, pHT1K-1Ac was introduced into *B. subtilis* isolate by electroporation and the transformant was confirmed by PCR with *cry1Ac* specific primers. *B. subtilis* transformant produced a parasporal inclusion in the cells as in *B. thuringiensis* and the size of that protein was appox. 130 kDa. The insecticidal activity of the transformant was checked against lepidopteran pest. This result suggests that this recombinant *B. subtilis* strain shows the possibility of controlling harmful insect pests as well as plant fungal diseases simultaneously at one crop or on industrial downstream, the culture broth and harvested cells can be used as individual biological control agents separately for integrated crop protection.

Poster / Microbial Control. Wednesday, 16:30. **MC-03**

Screening of *Bacillus thuringiensis* to the two-spotted spider mite *Tetranychus urticae*

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The two-spotted spider mite is the most important pest to brazilian papaya, specially at Espirito Santo State. The control of this pest by chemicals has increase the environmental problems and polution and besides this it has been observed higher rates of resistance evolution. The entomopathogenic bacterium *Bacillus thuringiensis* is the most succesfull wordwide biopesticide and in this study 48 *Bt* isolates were assayed against this pest. Suspensions with 3 x 10^8 spores.mL were offered to 80 *T. urticae* females (four replications) in *Canavalia ensiformes* foliar dishes. Two isolates, 689 and 980 were the most promissing ones causing mortality of 74.2% and 79.4%, respectively.

Poster / Microbial Control. Wednesday, 16:30. **MC-04**

Selection of *Bacillus thuringiensis* Cry toxins for the control of *Sitophilus oryzae* (Coleoptera: Curculionidae)

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This work was carried out to select Cry toxins against *Sitophilus oryzae*, the main brazilian pest of stored grains. First, 1073 brazilian *Bt* isolates were genetically characterized for the presence of *cry34* and *cry35* genes, reported elsewhere as toxic to coleopteran pests. The *cry34* gene was no observed, but the presence of *cry35* gene was verified in 60 isolates (5.6%). Four of them caused mortality above 50% in pathogenicity bioassays and the isolates 544 and 622 were the most virulent, as estimated by LC_{50} bioassays. By eletronic microscopy were observed spheric, cuboidal and bipiramidal crystals and by SDS-Page was possibile to verified the presence of 44 kDa proteins. This data shows the potential of *Bt* Cry toxins to manage this pest and further studies must be done to determine the strategy to use this entomopathogenic bacterium against this important pest, using as a biopesticide ou GM crops.

Poster / Microbial Control. Wednesday, 16:30. **MC-05**

Susceptibility of *Trichoplusia ni* (Lepidoptera: Noctuidae) to *Bacillus thuringiensis*

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The experiment was carried out at the Laboratory of Entomology of the Centro de Ciências Agrárias da Universidade Federal do Espírito Santo (CCA-UFES) to verify the susceptibility of *Trichoplusia ni* first instar larvae to 60 isolates of the entomopathogenic bacterium *Bacillus thuringiensis*. A suspension with 3 x 10^8 spores/mL was offered to 60 larvae and mortality was evaluated seven days after. The isolates E-1028, E-1050, E-996, E-921 and E-967, caused 100% of mortality and LC bioassays will be carried out to estimate the virulence of this pathogen to this important pest of soyabean and tomato in Brazil.

Poster / Microbial Control. Wednesday, 16:30. **MC-06 STU**

Effect of optical brighteners on the insecticidal activity of *Bacillus thuringiensis* ser. *kurstaki* and *Helicoverpa armigera* single nucleopolyhedrovirus

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The incorporation of certain stilbene optical brighteners into virus-based formulations has been demonstrated to increase viral pathogenicity (as indicated by reduced LD/LC_{50} values) but their effect on *B. thuringiensis* activity has been scarcely investigated. We determined the effect of nine optical brighteners on the insecticidal activity of *B. thuringiensis* ser. *kurstaki* HD-1 strain (Bt HD-1) on *Helicoverpa armigera* and also compared the effect of two optical brighteners on the insecticidal activity of Bt HD-1 and *H. armigera* single nucleocapsid nucleopolyhedrovirus strain SP1 (HearNPV-SP1). Blankophor CLE, Blankophor DRS, Blankophor ER, and Leucophor SAC significantly increased the pathogenicity of Bt HD1. In contrast, Tinopal UNPA-GX, Tinopal CBS, Blankophor BA, Leucophor AP, and Leucophor UO had an adverse or no effect on its activity. Tinopal LPW or Leucophor UO to HearNPV-SP1 significantly enhanced viral pathogenicity by 31.4 and 11.4 fold, respectively, when used at 1% and by 11.4 and 6.3 fold, respectively at 0.1%. However, none of these brighteners increased Bt-HD1 activity. We hypothesize that the degradation of the peritrophic membrane by optical brighteners causes the enhancement of HearNPV pathogenicity and the null or antagonistic activity on Bt HD-1 against *H. armigera*. Inclusion of optical brighteners on Bt-based formulations is discussed.

Poster / Microbial Control. Wednesday, 16:30. **MC-07**

Future potential for biological control of *Neodiprion sertifer* Geoffr. and *Bupalus piniarius* L. in Latvia: occurrence and variability of pathogens

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The European pine sawfly *Neodiprion sertifer* (Geoffr.) and pine looper *Bupalus piniarius* L. are often present in the pine stands in western part of Latvia. It is now widely appreciated that the safe and effective use of entomopathogens requires a greater knowledge of how these pathogens behave in natural populations of insects. The main tasks of our studies were: 1) to obtain new isolates of pathogens and to describe their morphological and biological characteristics, 2) to describe the natural occurrence of pathogens in pest populations. New sensitive methods of pathogen detection are used for monitoring of occurrence and presence of pathogens in the insect populations. We observed territories in western part of Kurland covered by outbreak of *N. sertifer* and *B. piniarius.* High level of defoliation was observed in inspected pine stands.We isolated nuclear polyhedrosis viruses from *N. sertifer* and *B. piniarius* populations. Granulosis virus was isolated from *B. piniarius*. Entomopathogenic fungi *Beauveria bassiana* was isolated from cocoons of both pests. This work has been financially supported by the grant from the Foundation of Forest Development.

Poster / Microbial Control. Wednesday, 16:30. **MC-08**

Searching for pathogens to control stored product mites (Acari: Acaridida)

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Stored product mites represent a serious pest due to their contamination of human food and animal feed by allergens. There is a strong effort in eliminating stored product mite infestation to zero or sub-zero level. The public demand for elimination of pesticides from the food industry for their negative effects on human health and the environment limited chemical pesticides application. Microbial pathogens and viruses toxic to mites are suggested as an alternative to chemical pesticides. We summarize the results of testing *Bacillus thuringiensis* toxins on acaridid mites. In addition we report the presence of gram negative proteobacteria that act as entomopathogenic symbionts in the digestive tract of nematodes (*Steinernematidae* and *Heterorhabditidae*). The clones of 16S rRNA genes from stored product mite homogenates showed high similarity to *Xenorhabdus cabanillasii* and 91% similarity to *Photorhabdus temperata*. In midgut cells of stored product mites, the unidentified icosahedral viral particles were observed. Many viral particles were found in the postcolon inside the microvilli and formed chain-like structures. The potential of abovementioned bacteria and viruses in control of stored product mites are discussed. This work was supported by the projects COST OC08065 and NAZV 1B 53043.

Poster / Microbial Control. Wednesday, 16:30. **MC-09**

Microbial control of insect pests in temperate orchard systems: Status and future prospects

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Due to their selectivity and safety, microbial control agents (MCAs) are ready made components of IPM systems that will not pose a threat to applicators or the environment. Control of several orchard pest insects using MCAs, including viruses, *Bacillus thuringiensis*, fungi and entomopathogenic nematodes have been demonstrated in apple, pear, stone fruits, citrus and several nut crops. *Bacillus thuringiensis* is the most used MCA for control of lepidopteran orchard pests. Significant use of entomopathogenic nematodes in citrus for control of root weevils is also reported. The granulovirus of codling moth is increasingly being used in apple and pear by organic growers with interest also shown by conventional growers. We conclude that integrating MCAs into orchard IPM will have minimum impact on the actions of other natural enemies, and that comparison of MCAs with broad spectrum chemical pesticides

should not be made strictly on a cost and efficacy basis but also on the spectrum of beneficial properties provided by MCAs. We predict that an increase in the efficacy of MCAs will be fostered through discovery of new strains, improvement of existing strains through molecular and non-molecular methods, superior application procedures, and improvement of environmental persistence through formulation and environmental manipulation.

Poster / Microbial Control. Wednesday, 16:30. **MC-10**

Biological control of the fall webworm, *Hyphantria cunea* (Lepidoptera: Arctiidae) using a complex of entomopathogenic agents in Georgia

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The fall webworm, *Hyphantria cunea (Lepidoptera: Arctiidae)* is dangerous quarantine pest damaging the agriculture crops, forest and ornamental plants, it distributed through the territory of West Georgia and Black Sea Coast. The insect mainly inhabits in the populated area – home sites, communities suburban parks and places of mass restoring where by the viewpoint of sanitation-hygiene the using chemical pesticides are prohibited. Generally the urban horticulture is under the threat of fall webworm. In this connection it is necessary the use of environmentally safe means to plant protection from this dangerous pest. The biological control potential of different means: bacterial - *XenTari DF, Dipel,* fungal - *BotaniGard ES* (Project – GRDF-GEB2-3337-TB-04, USA) and entomopathogenic nematode (EPN) introduced from Israel (CDR-CAR Project CA CA22-007) – *Steinernema feltiae* were tested against 2nd, 3rd instars larvae and pupae of *H.cunea* in laboratory and fields. Infectivity of *H.cunea* by the suspensions (0.7%) *XenTari DF* and *BotaniGard ES* on 7 days have caused 96-100% mortality. The mix infection at the reduced concentrations of microbial means with entomopathogenic nematodae, *S.feltiae* (1.500 unit/ml) on 3 days has caused 100% mortality of larvae, which may serve for cultivation of nematodes. Biological control will take the important place in IPM.

Poster / Microbial Control. Wednesday, 16:30. **MC-11**

Potential for entomopathogens against invasive species in landscape ornamentals in Florida

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Florida is particularly prone to invasions from non-indigenous insect species. The absence of yearly hard freezes (most of the state has a climate similar to that of the Neotropics), an impoverished native flora and fauna and a diverse patchwork of agricultural, environmental, aquatic and urban habitats presents many opportunities for the establishment of alien species. While this state has been invaded by tropical and subtropical species for over 400 years, introductions have expanded during the twentieth century – coinciding with the huge growth of the ornamental plant industries and unintentional contaminants of imported commodities. Managed landscapes with diverse ornamental plantings also provide corridors through which recent invasive species may move rapidly. While chemical insecticides remain the first choice of most landscape managers, such approaches only provide short term relief and are often at conflict with the longer term environmental goals, especially in urban areas. Work starting at the Mid-Florida Research & Education Center in 2008 aims to evaluate the potential of a number of entomopathogens to controlling a range of recent invasive pests of landscape ornamentals. Initial projects will focus on the pink hibiscus mealybug, *Maconellicoccus hirsutus , and chilli thrips, Scirtothrips dorsalis Hood.*Green

Poster / Microbial Control. Wednesday, 16:30. **MC-12**

Alkane-growth adaptation enhances virulence of *Beauveria bassiana* against *Triatoma infestans*, the major Chagas disease vector in Argentina

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The insect cuticle is the first barrier against biological or chemical contact insecticides. A thin layer of lipids, mainly hydrocarbons, protects insects against lethal desiccation; they are proposed as a new target for triatomine control. We studied the effect of alkane-growth adaptation of the entomopathogenic fungus *Beauveria bassiana* on the ability to infect the Chagas disease vector *Triatoma infestans*. The bioinsecticide capacity of two fungal strains (Bb GHA and Bb 10) was compared in fungi grown in two different carbon sources (glucose and insect-like hydrocarbons) as the sole carbon source. Mortality and median lethal time were evaluated at different doses (10^7, 10^8, and 10^9conidia/ml). The alkane-grown fungi showed enhanced virulence parameters. An increased mortality percentage (>50%) and/or a significant reduction (>15%) in the time to kill *T. infestans* were observed, compared to controls grown in complete medium. These evidences suggest that the initial steps of infection might be favored by using an insect-host hydrocarbon mimic as the sole carbon source for fungal growth.

Poster / Microbial Control. Wednesday, 16:30. **MC-13**

Effect of formulating of *Beauveria basssina* conidia on their viability and pathogenicity against the onion thrips, *Thrips tabaci*

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Wettable powders were prepared on the basis of aerial conidia for two isolates of *Beauveria bassiana*. Conidia viability and pathogenicity were evaluated against second-instar larvae of onion thrips, *Thrips tabaci* in four cases; Conidial-product Maintained in Refrigerator (CMR),Conidial- product Maintained in Laboratory (CML), New Formulated Conidia (NFC) and New Conidia without formulation (NC). Analysis of corrected seven-day total mortality data demonstrated that there are significant differences among these product-cases in their pathogenicity against thrips larvae. Recorded mean mortality rates for CMR, CML, NFC and NC after treatment with 10^8conidia/ml were 48, 63, 67 and 67% for *B. bassiana* EUT105 and 45, 63, 76 and 75% for *B. bassiana* EUT116, respectively. In the next step, salt components ($MgCl_2$, NH_4PO_4, KH_2PO_4, $MgSO_4$ and NaCl) were added at a rate of 0.1 M into the both CMR and CML products. Bioassay results indicated that caused total mortalities on thrips larvae were increased with adding of salts. Mortality rates of second-instar larvae for CMR-S and CMR were 94 and 63% in *B. bassiana* EUT105 and 89 and 63% in *B. bassiana* EUT116, respectively. Similarly, recorded mortality rates on thrips larvae for CML-S and CML were 62 and 48 % in *B. bassiana* EUT105 and 63 and 45 % in *B. bassiana* EUT116, respectively. In general, our results demonstrated that applied carriers and salt components have positive effects on preservation of conidia viability and pathogenicity against second-instar larvae of the onion thrips.

Poster / Microbial Control. Wednesday, 16:30. **MC-14**

Incidence, persistence and efficacy of *Beauveria bassiana* in cherry orchard soils

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In the Pacific Northwest of North America, western cherry fruit fly, *Rhagoletis indifferens* (Diptera: Tephritidae) drop from infested fruit as late instar larvae and overwinter as pupae in the orchard soil. Both the larvae and pupae are susceptible to infection by *Beauveria bassiana*-GHA and the isolate could potentially be used as a bioinsecticide versus this important cherry pest. A soil survey of organically and chemically managed cherry orchards in southern British Columbia, Canada was conducted to determine the incidence of indigenous *B. bassiana* isolates. When the same soils were tested for their ability to support introduced Bb-GHA over time, the persistence of conidia germination in non-sterile soils was variable for four weeks post-treatment. The efficacy and logic of using Bb-GHA to cause western cherry fruit fly mortality within orchard soils was evaluated.

Poster / Microbial Control. Wednesday, 16:30. **MC-15 STU**

SEM study of the infection of the red palm weevil *Rhynchophorus ferrugineus* by *Beauveria bassiana*

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Palms are an important crop in Mediterranean zone and in dry areas worldwide. The red palm weevil (RPW, *Rhynchophorus ferrugineus*) was introduced in Europe with N Africa importations of infested plants fifteen years ago. Ineffective control measures (repeatedly applied chemicals have been futile for RPW control) against RPW are causing both severe economic and environmental damages. Consequently, there is a need for a Sustainable Pest Control of RPW. Infection of RPW adults and larvae by strains of the entomopathogenic fungus *Beauveria bassiana*, recently isolated from naturally infected RPW in the field, was investigated using Scanning Electron Microscope (SEM). RPW adults were treated with dry conidia and diluted conidia in 0.02% Tween-20. More apresoria were observed in the treatment of adults with dry conidia than in diluted conidia. Apresoria were also observed in larvae treated with dry conidia. The obtainment of fungal inoculum became favoured by different structures of the insect. These results suggest several ways of inoculum acquisition by the insect. This will help to fully develop *B. bassiana* as a biological control agent of RPW.

Poster / Microbial Control. Wednesday, 16:30. **MC-16**

Use of *Beauveria bassiana* as a tool for biological control of *Rhynchophorus ferrugineus*

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The red palm weevil *Rhynchophorus ferrugineus* (RPW) is a serious pest of date palm (*Phoenix dactylifera*) and other Palmaceae in Spain and other Mediterranean countries. Recent outbreaks of RPW in Spain promted approaches to control the pest. Chemical control approaches of RPW in Spain have proven inefficient. As a consequence, RPW first detected in Granada (southern Spain) it has expanded throught the Spanish Mediterranean zone. We reported natural infection of RPW with the entomopathogenic fungus *Beauveria bassiana* in SE Spain. This suggested the development of a biocontrol strategy of RPW using the fungus. For this purpose, pathogenicity of 9 *B. bassiana* strains (including RPW isolates) was evaluated in laboratory bioassays with RPW larvae. LT_{50} values of the strains were between 3-10 days. The best *B. bassiana* isolates were tested in RPW adults and these were formulated for use against RPW. These results suggest that *B. bassiana* has the potential to be developed in a biological control strategy of RPW. Furthermore they also show that the choice of the adequate strain is a key step in the development of an efficient biocontrol agent for RPW.

Poster / Microbial Control. Wednesday, 16:30. **MC-17**

Pathogenicity of *Beauveria bassiana* and *Cladosporium cladosporioides* to the two-spotted spider mite *Tetranychus urticae*

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The two-spotted spider mite is the most important pest to brazilian papaya, especially at Espirito Santo State. The control of this pest by chemicals has increase the environmental problems and polution and besides this it has been observed higher rates of resistance evolution. The entomopathogenic fungi *Beauveria bassiana* and *Cladosporuim cladosporioides* were assayed against this pest as an alternative to chemical control. Conidial suspensions (10^8 conidia.mL) were prepared and offered to ten females in *Canavalia ensiformes* foliar dishes. There was significantly differences between the isolates and fungi assayed. Mortality caused by *B. bassiana* ranged from 59.0% to 88.3% and to *C. cladosporioides* the mortality ranged from 66.7% to 71.3%. Both fungi are promissing biological control agents for the control of *T. urticae* in brazilian papaya.

Poster / Microbial Control. Wednesday, 16:30. **MC-18 STU**

Occurrence and distribution of *Beauveria* and *Metarhizium* in Moroccan soil

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The fungi *Beauveria* and *Metarhizium* are world-wide distributed. These species are frequently found in the soil and have been known by their ability to control a wide range of insects. The use of local Entomopathogenic fungi will be appropriate to control insects since these strains are adapted to biotic and abiotic factors. The aims of our investigation is to analyze the occurrence and the natural distribution of *Beauveria* and *Metarhizium* in Moroccan and to isolate and select potential strains to control insects which cause important losses to Moroccan economy. The presence of entomopathogenic fungi was examined by using selective medium of *Beauveria* and *Metarhizium* and by *Galleria baiting* methods. Approximately 55% of soil samples have shown the occurrence of *Beauveria* on selective medium. However, Metarhizium have found at a low rate. The total number of *B.brongniartii* CFU is approximately identical to *B.bassiana* ones. The baiting method revealed the presence of Entomopathogenic fungi in all soil samples. More than 400 isolates were identified and stored. *Beauveria* are predominant in all soil samples, whereas *Metarhizium* were found at a lower occurrence. Nevertheless, the *Metarhizium* are recovered mainly from the south-western soil samples. These isolates constitute the first Moroccan collection of Entomopathogenic fungi. The molecular studies are underway in order to analyze their diversity.

Poster / Microbial Control. Wednesday, 16:30. **MC-19**

Evaluation of *Metarhizium anisopliae* for wireworm control in Switzerland

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Indigenous isolates of the entomopathogenic fungus *Metarhizium anisopliae* were screened for virulence against the wireworm species *Agriotes obscurus*, *A. lineatus* and *A. sputator*. In 2006, thirteen isolates were tested by dipping larvae into blastospore suspensions ($1x10^7$ spores/mL). For the most virulent isolates, a maximum of 38.3%, 30.0%, and 30.4% of the *A. obscurus*, *A. lineatus* and *A. sputator* larvae, respectively, were infected after nine weeks. In 2008, a similar bioassay was performed using conidial ($1x10^7$ spores/mL) instead of blastospore suspensions. For the most virulent isolates infection rates were 80.0 %, 33.3%, and 40.0% for *A. obscurus*, *A. lineatus* and *A. sputator* larvae, respectively, after nine weeks. The results suggest that the *M. anisopliae* isolates may be particularly useful to control *A. obscurus*, while control of the other two wireworm species may be less efficient.

Poster / Microbial Control. Wednesday, 16:30. **MC-20 STU**

Evaluating bioassay techniques for infection of *Rhipicephalus* ticks (Acari: Ixodidae) with entomopathogenic fungi

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Strain selection is a critical step towards the successful development of a mycoinsecticide and depends heavily on sound bioassay procedures. This study examined the strengths and weaknesses of bioassays employed for screening of fungal isolates that are pathogenic to ticks and seek to identify the most suitable technique for infecting *Rhipicephalus* ticks in the laboratory. Three techniques, namely Burgerjon's spray tower, immersion and microinjector were evaluated for inoculating adults of *Rhipicephalus pulchellus (Acari: Ixodidae)* with *Metarhizium anisopliae* (icipe 60) suspension containing 10^9 conidia/ml formulated in sterile distilled water, emusifiable oil and oil. Tick mortality in the test treatments and compatibility with the various conidial formulations were used as criteria for selecting the most appropriate technique for inoculation of ticks with entomopathogenic fungi. The least tick mortality (1.7%) was recorded in microinjector inoculation technique for aqueous formulation at 3 weeks post treatment. High tick mortality (84.2%) was caused by conidia in emulsifiable formulation in Burgerjon's spray tower, and the result was not significantly different ($P > 0.05$) from the widely used emersion technique (99.1%). Based on compatibility with the formulations and tick mortality, Burgerjon's spray tower was identified as the most suitable technique for inoculating adults of *Rhipicephalus* ticks in the laboratory.

Poster / Microbial Control. Wednesday, 16:30. **MC-21**

Evaluation on the potential of native fungal isolates against the Mexican bean bruchid, *Zabrutes subfaciatus* (Coleoptera: Bruchidae) in Ethiopia

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The virulence of five isolates of the entomopathogenic fungus Metarhizium were evaluated against Zabrotes subfaciatus (Boheman) (Coleptrea: Bruchidae) under laboratory conditionss. Four native isolates, META-B, META-D, PPRC-6, PPRC-29, and one standard isolate, ICIPE-30 were applied at different concentrations of conindia/ml. The experimental bruchids were treated by sparaying 1ml of ecah fungal concentration. All the isolates tested were found to be virulent at different magnitudes based on level of conidial concentrations. Among the Metrahizium isolates, META-D and ICIPE-30 were proved to be best performing in ther virulence compared with other isolates tested. In most post treatement days, a significant difference ($P<0.05$) in mortality was observed between fungal concentrations (doses) and the control. Isolate META-B was found to be the least performing isolate with ca. 67.5% target mortality with confirmed symptoms of mycosis. The present study suggests that the use of entomopathogenic fungi may hold promise as an alternative approch to chemical insecticides against one of the major sorage insect pests, the Mexican bean bruchid. Discussions in this presentaion are expected to to contribute towads expereince sharig in advancing the development and application of myco-insecticides for stored product pests in general.

MICROSPORIDIA

Poster / Microsporidia. Wednesday, 16:30. **M-01**

Vairimorpha invictae not detected in the parasitic fly, _Pseudacteon obtusus_, reared from the microsporidium-infected fire ants, _Solenopsis invicta_

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The microsporidium *Vairimorpha invictae* and parasitic flies in the genus *Pseudacteon* have established or are being considered for release in the USA for the biological control of fire ants, *Solenopsis invicta*. *Pseudacteon* flies oviposit into adult fire ants, where maggots that eclose from eggs migrate to the ants' head, pupate, and eventually decapitate the host. The compatibility of these biocontrol agents was examined by determining if the parasitic fly, *P. obtusus* would become infected if it developed in the microsporidia-infected fire ants. *P. obtusus* were allowed to oviposit and develop in *V. invictae* infected *S. invicta* in the laboratory. There was no evidence of microsporidian infection in *P. obtusus* adults that developed in heads that were matched to infected bodies (n=39). *S. invicta* bodies that could not be matched with their decapitated heads had an estimated infection rate of 87%. *V. invictae* was not detected in any of the *P. obtusus* that emerged from unmatched heads (N=318). These results further defined the host specificity of *V. invictae* and indicated that *V. invictae* will not directly interfere with *P. obtusus* parasitism.

Poster / Microsporidia. Wednesday, 16:30. **M-02**

A new _Cystosporogenes_ isolate from _Agrilus anxius_ (Coleoptera: Buprestidae)

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We isolated and characterized a new microsporidium from the bronze birch borer, *Agrilus anxius* Gory. All stages including mature spores lay in vacuoles bounded by a single membrane which was in contact with the host cell cytoplasm. The mature spores of this new isolate are monokaryotic and measured 2.60 (±0.12) × 1.66 (±0.12) µm (*n*=50). The ultrastructural and spore morphological features indicate that this microsporidia possesses the entire dichotomous key of the genus *Cystosporogenes*. There was a 99% nucleotide sequence identities between the described isolate and other members of the genus *Cystosporogenes*. Phylogenetic analyses based on the small subunit rRNA gene also placed the new isolate in the *Cystosporogenes* cluster which further confirmed the classification of the new isolate. Prevalence was very high (up to 85%) at the localities for the three years sampled. Infection rate was higher in late than early emergent beetles but no sex dependent differences in infection could be observed.

Poster / Microsporidia. Wednesday, 16:30. **M-03**

Modeling horizontal transmission of microsporidia in _Lymantria dispar_

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Lymantria dispar, a well-known defoliator of broadleaved forests, is host for a variety of entomopathogenic microsporidia. In a series of laboratory and semi-field experiments, we studied horizontal transmission of *Endoreticulatus schubergi*, a midgut parasite, *Vairimorpha disparis*, a fat body parasite, and *Nosema lymantriae* a parasite causing systemic infections. Despite the fact, that the three species infect different target tissues, only two main horizontal transmission pathways were identified. *Endoreticulatus schubergi* is transmitted via spore-laden feces, *V. disparis* via decomposing cadavers; *N. lymantriae* uses both transmission pathways. We developed a simulation model that describes stage specific development and mortality of uninfected, latent and infectious hosts and the two main transmission pathways. Results of the laboratory experiments were used to calculate parameters and fit equations for spore release via feces and survival of hosts, which both served as input variables. The number and percentage of infected larvae served as output variable and was compared to the results of the semi-field studies.

NEMATODES

Poster / Nematode. Wednesday, 16:30. **N-01**

Hexamermis sp. an entomopathogenous nematode associated with the European stink bug

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The nematodes of the Mermithidae family are a large and important group obligatory parasite of arthropods, principally insects, and are almost always lethal to their hosts. They are usually specific to a single species or to one or two families of them. A mermithid of the genus *Hexamermis* Steiner, was found parasiting the stink bug *Rhaphigaster nebulosa* Poda (Hemiptera: Pentatomidae), present on hazelnut plants in the Piedmont region. The bug feeds on various broadleaved woody plant and is considered a serious pest for hazelnut in Italy. Considerations regarding the taxonomy of the *Hexamermis* genus are reported. From the taxonomic point of view is very difficult to describe the different species of *Hexamermis*. Morphologically they are similar but the biology and the ecology of all these species is almost unknown. For this reason it is difficult to identify with a certain degree of precision the species. The specimen found, from the morphological data, is probably *H. albicans*. This findings is particularly interesting as there is restricted literature about the mermithids which attack Rhynchota. Moreover there are few mermithids of this genus reported from Hemiptera. Further investigations are necessary to better understand the taxonomy and biology of this mermithid and to know its role as a biological agent in controlling this or other bugs of hazelnuts.

Poster / Nematode. Wednesday, 16:30. **N-02**

Habitat complexity effects on movement of *Steinernema carpocapsae* in maize

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Habitat heterogeneity enhances the conservation of aboveground biological control organisms, but this has rarely been examined for soil organisms. We compared the effect of simple (maize) and more complex (maize plus mixed annual plant refuge) habitats on the persistence and dispersal of the Steinernema carpocapsae applied to soil as infected insect cadavers. We quantified S. carpocapsae dispersal by bioassay of soil samples collected at distances up to 3m away from the application point within and between crop and refuge habitats. Detection of S. carpocapsae at the source was associated with soil bulk density, plant density, and soil matric potential, but not habitat complexity. The maximum movement rate was 33.3 cm/day, which exceeded previously reported rates of 7.5cm/day. In 2005, soil moisture had the largest effect on dispersal with S. carpocapsae detected further away in complex habitats, when the soil moisture in this habitat was higher. In 2006, movement was similar in both habitats, which was likely due to similarities in overall plant density. Our results indicate that movement of S. carpocapsae is not necessarily dependent on plant diversity, but may respond to variation in factors associated with overall plant density, and subsequently, soil moisture.

Poster / Nematode. Wednesday, 16:30. **N-03**

Pathogenicity of *Thripinema fuscum* Tipping & Nguyen (Tylenchida: Allantonematidae) infecting *Frankliniella fusca* (Hinds) (Thysanoptera: Thripidae)

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The insect parasitic nematode *Thripinema fuscum* is a key regulator of *Frankliniella fusca* in agricultural peanut across the southeastern United States. Parasitism by *T. fuscum* causes a significant reduction in both the feeding and fecundity rates of adult female thrips, and as a result, reduces the vector competence (acquisition and transmission) of *F. fusca* to spread Tomato spotted wilt virus. The potential of *T. fuscum* to act as a biological control agent of *F. fusca* has been recognized; however, very few studies have investigated the pathological changes induced by the entomogenous parasite. Future elucidation of the mechanisms responsible for shutting off egg production in parasitized thrips may provide novel avenues for regulating the intrinsic rate of increase of this pest insect. Understanding the mechanism(s) leading to reduced Tospovirus competency in parasitized thrips may also provide targets that suppress disease spread. To determine how the parasitic *T. fuscum* modulates the physiology of the thrips host and how such alterations influence vector competence, the impact of *T. fuscum* on host thrips was examined using a combination of light and electron microscopy. Changes to *F. fusca* tissues affected by *Thripinema* invasion and replication were recorded and a possible explanation of the cause provided.

Poster / Nematode. Wednesday, 16:30. **N-04 STU**

Susceptibility of the Colorado Potato Beetle to the nematode *Pristionchus uniformis*

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Pristionchus pacificus is a diplogastrid nematode used as a model organism in comparative developmental and genetic studies. *P. pacificus* and other members of this genus can live in soil as well as associated with beetles. Previous studies have shown a nearly species-specific host association (e.g. *P. pacificus* occurs mainly on the Oriental Beetle *Anomala orientalis* and *P. uniformis* on the Colorado Potato Beetle *Leptinotarsa decemlineata* (CPB)). The *Pristionchus*-beetle association is thought to be necromenic. The dauer larvae attach to the host and remain there until the insect dies whereby the feed on bacteria and nematodes growing on the carcass. *P. uniformis* however has been shown to infect and kill CPB, a global pest of potato crops. Hence we decided to investigate this relationship further in order to provide a quantitative assessment of the *Pristionchus*-beetle association. First, we artificially infected CPB pupae with *P. uniformis* dauer larvae and determined the nematode load as well as the host mortality. Second, we tested bacterial strains isolated from natural populations of *P. uniformis* for their role in the infection process. Finally, we analyzed wild caught CPB by high throughput sequencing analysis to determine natural infection levels.

OTHER

Poster / Other. Wednesday, 16:30. **O-01**

Toxicity of azadirachtin and some of its molecule analogue portions on larvae of *Galleria mellonella* (Lepidoptera) and on insect cell cultures

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Investigations of toxicity of simpler molecules based on the epoxy-alcohol fragment of azadirachtin have revealed insecticidal activity on *Galleria mellonella* L. larvae. The epoxy-alcohols doses giving 50% mortalities were in the increasing order from 2,3-epoxy-1-propanol (I) (0.22 mg/g), 4.5-epoxy-2-pentanol (II) (0.068 mg/g) and finally, 1,3-bis(2,3-epoxy-1-propyloxyl)-2-propanol (III) (0.147 mg/g). The epoxyalcohols exhibited higher insecticidal activity when compared with the commercial neem product for which the dose giving 50% mortalities was 10.6 mg/g and to azadirachtin that killed the larvae only by injection (dose of 0.20 mg/g of body weight). Following the results of these *in vivo* experiments, we tested them *in vitro* on insect cell lines. First, we described the cytotoxic effects on three cell lines (Sf9, A.t. GRIP-1, and Ld-L1) of (I), (II), (III), 3-epoxy-1-propyl butyrate (IV), 2,3-epoxypropyl methyl ether (V), azadirachtin and a neem formulation. We observed, depending on the concentrations, immediate and delayed cytotoxic effects. All molecules tested blocked cell proliferation to the same extent on Sf9 cell line. An antimitotic effect was observed with azadirachtin and the molecules tested on Ld-L1 cells. The cytotoxicity of our molecules emphasizes the importance of the chemical structure between the two half moieties of azadirachtin for the biological activity.

Poster / Other. Wednesday, 16:30. **O-02**

Cloning and expression of a venom protein from the endoparasitoid, *Pimpla hypochondriaca*, which has haemocyte anti-aggregation activity *in vitro*

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Venom from the endoparasitoid, *Pimpla hypochondriaca* contains a mixture of proteins. One of these was previously biochemically isolated and shown to have haemocyte anti-aggregation activity against host haemocytes *in vitro*. This protein shares significant homology to a second venom protein (VPB) from this parasitoid. The gene for VPB was amplified from a *P. hypochondriaca* venom gland cDNA library by PCR, cloned and expressed in *E. coli*. The presence of a fusion tag allowed purification. The purified immunosuppressive protein was found to have anti-haemocyte activity, *in vitro*, against haemocytes from two lepidopteran pests. This venom protein may have the potential to improve efficacy of biocontrol agents.

Poster / Other. Wednesday, 16:30. **O-03**

A recombinant immunosuppressive protein from *Pimpla hypochondriaca* increases the susceptibility of two lepidopteran pests to *Bacillus thuringiensis*

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Venom from the endoparasitic wasp, *Pimpla hypochondriaca*, contains factors with anti-haemocyte and immunosuppressive properties. The gene for one such factor (*vpb*) has been cloned and recombinant protein produced. Bio-assays utilising VPB were performed and indicated that introduction of this immunosuppressive protein into the haemocoel of two lepidopteran pests, increases their susceptibility to the biological control agent, *Bacillus thuringiensis*. The potential for improving the efficacy of *Bt* through suppression of pest immune responses is discussed.

VIRUSES

Poster / Virus. Wednesday, 16:30. **V-01**

Characterization of white spot syndrome virus envelope protein VP51A and its interaction with viral tegument protein VP26

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Temporal transcription analysis showed that white spot syndrome virus (WSSV) *vp51A* is a late gene. Gene structure showed the transcription initiation site of *vp51A* was 135 bp upstream of the translation start codon. The poly-A addition signal overlapped with the translation stop codon TAA, and the poly-A tail was 23 bp downstream of the TAA. Western blot analysis of viral protein fractions and immunoelectron microscopy both suggested that VP51A is a viral envelope protein. Western blotting of the WSSV virion total proteins detected a band that was close to the predicted 51 kDa size, but the strongest signal was around 72 kDa. This 72 kDa band appeared to be the full length VP51A protein, and we hypothesize that the smaller bands (51 kDa, 43 kDa and others) were the result of post-translational processing. The apparent MW of the 72 kDa band may have been due to the large proportion (23%) of charged residues. Membrane topology assays demonstrated that VP51A is a type II transmembrane protein with a transmembrane domain on its N-terminal. Co-immunoprecipitation and co-localization assays revealed that VP51A associated directly with VP26 and indirectly with VP28, with VP26 acting as a linker in the formation of a VP51A-VP26-VP28 complex.

Poster / Virus. Wednesday, 16:30. **V-02**

Transactivation, dimerization, and DNA-binding activity of WSSV immediate early protein IE1

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Here, we investigate transactivation activity, DNA binding activity and dimerization in white spot syndrome virus (WSSV) immediate early protein 1, IE1, and attempt to map the corresponding functional domains. Transactivation was investigated by using transiently expressed GAL4-IE1 fusion proteins to drive baculovirus *Autographa californica* multiple nucleopolyhedrovirus *p35* basal promoter with five copies of the GAL4 DNA binding site upstream of the TATA box. A deletion series of GAL4-IE1 fusion proteins suggested that the transactivation domain of WSSV IE1 was encoded within aa 1-80. Point mutation further showed that all twelve of the acid residues in this highly acidic domain were important for IE1's transactivation activity. DNA binding activity was confirmed by an electrophoresis mobility shift assay using a probe with ^{32}P-labeled random oligonucleotides. The DNA binding region of WSSV IE1 was located in its C-terminal (aa 81-224), but mutation of a putative zinc finger motif in this region suggested that this motif was not directly involved in the DNA binding activity. A homotypic interaction between IE1 molecules was demonstrated by GST pull-down and a coimmunoprecipitation analysis. A glutaraldehyde cross-linking experiment and gel-filtration analysis showed that this self-interaction led to the formation of stable IE1 dimers.

Poster / Virus. Wednesday, 16:30. **V-03**

Characterization of the *Amsacta moorei* entomopoxvirus spheroidin promoter

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Spheroidin (SPH) is the most abundantly expressed protein in the *Amsacta moorei* entomopoxvirus (AMEV). The *sph* promoter contains a conserved TAAATG motif typical to late poxvirus genes, which serves as the initiation site for both transcription and translation. Additional downstream sequences are also involved in the high expression of *sph*. In an effort to characterize the *sph* promoter, we used a transient assay expressing luciferase in constructs containing 160 bp of the upstream region which included the conserved motif, and up to 103 bp from the downstream *sph* ORF. The constructs containing 4 – 25 bp of *sph* ORF showed optimum expression from the promoter, while adding more than 25 bp of downstream sequences led to a reduction of luciferase expression. Next, we analyzed the upstream promoter region using constructs with 3 bp of sph ORF (included in TAAATG motif) and different lengths of upstream sequences. We showed that at least 160 bp of the upstream region is required for optimum expression, but a minimum of 40 bp is sufficient for low level expression.

Interestingly, further upstream sequences significantly inhibited the activity of the promoter. We are currently investigating the presence of any regulatory elements within the upstream region.

Poster / Virus. Wednesday, 16:30. **V-04**

Effects of chitinase J on the insecticidal efficacy of *Autographa californica* multiple nucleopolyhedrovirus

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Chitinase has developed as the biological insecticide since the 1990s; it is effective to degrade the institutional framework of insect and then cause insect death. As far as it is known that the chitinase from insect has the best insecticidal efficacy among the different sources of chitinase. Relatively, the insecticidal efficacy is not well when the chitinase from bacterium. On the other side, the insecticidal efficacy of chitinase from the plant is not yet studied. Thus, we study the effects of chitinase J (Chi J) from Jelly Fig (*Ficus awkeotsang*) on the insecticidal efficacy of *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV). The chi J is a 45 kDa protein, belongs to family 18 of the glycohydrolase superfamily. In this study, the third instar of *Trichoplusia ni* larvae were treated by the 1mg/ml of Chi J combined with $1x10^8$ PIBs/ml of AcMNPV. Results showed that the LD_{50} value was reduced from $1.46\ x10^6$ PIBs/ml to $1.1\ x10^3$ PIBs/ml. Further, we employed the internal ribosome entry site (IRES) element of *rhopalosiphum padi* virus (RhPV) to construct $pAcP_{10}$ChiJ-IR-EGFP for Chi J and EGFP genes expression. We expect that the polyhedrin-positive recombinant baculovirus $vAcP_{10}$ChiJ-IR-EGFP will enhance the insecticidal efficacy of baculovirus in infected *Trichoplusia ni* larvae.

Poster / Virus. Wednesday, 16:30. **V-05**

Reprogramming expression of chitinase and cathepsin of the *Autographa californica* multiple nucleopolyhedrovirus

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Reprogramming for increased expression of baculovirus chitinase and cathepsin using native baculovirus promoters might provide a platform for designing environmentally benign biological insecticides. To establish a baseline for the recombinant AcMNPV studies, we first monitored native temporal *chiA* and *v-cath* transcription. Replacing 21 nucleotides containing the native late promoters in the *chiA/v-cath* intergenic region with a selectable *polh-EGFP* cassette was sufficient to abrogate both *chiA* and *v-cath* transcription. Exchanging EGFP with either the *p6.9* or *polh* promoters to drive *chiA* transcription produced marked differences in the temporal *chiA* transcription profiles and also increased CHIA enzyme activities by 3 or 4 fold at 48 h.p.i. relative to that from the native promoter. Transcription of *v-cath* was detectable by 9 h.p.i., but *v-cath* RNA or enzyme expression was undetectable through 48 h.p.i. in the *chiA*-reprogrammed viruses bearing a reconstituted native-like *v-cath* promoter. However, by a dual reprogramming of expression of *chiA* with the *p6.9* promoter and *v-cath* with the *polh* promoter, both CHIA and V-CATH enzyme production were rescued. Furthermore, preliminary data indicated there was an increase in levels of both enzymes due to the dually reprogrammed transcription of each gene from the alternate promoters.

Poster / Virus. Wednesday, 16:30. **V-06**

Transactivation of *Epinotia aporema* granulovirus (EpapGV) promoters in *Anticarsia gemmatalis* cells

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Epinotia aporema (Lep. Tortricidae) and *Anticarsia gemmatalis* (Lep. Noctuidae) are major pests of legume crops in South America. On many occasions they are found simultaneously in the same fields. A granulovirus (EpapGV) characterized in our laboratory exhibits a great potential as bioinsecticide and AgMNPV has been successfully used in Brazil. In more temperate climats the insecticidal activity of both viruses needs improvement in order to compete with chemical pesticides. To this end we developed a system to genetically improve AgMNPV taking advantage of the availability of the susceptible cell line UFLAg-286 and speculated about eventually extending its host range by inserting large segments of EpapGV DNA. One condition for this strategy is that the EpapGV promoters should be active in the heterologous context of AgMNPV-infected *Anticarsia gemmatalis* cells, that are not permissive for infecction by this GV. To evaluate this hypothesis we transfected UFLAg-286 with plasmid constructs containing the *E. coli lacZ* under the control of various EpapGV and AgMNPV promoters. Our results indicate that *ie1* promoter of either virus enables expression of *lacZ* in the absence of any viral factor and that transcription driven by EpapGV late and very late promoters can be transactivated by heterologous gene products during AgMNPV infection.

Poster / Virus. Wednesday, 16:30. **V-07 STU**

Early gene *hhi1* of HzNV-1 virus is a strong apoptosis inducer and crucial for latent viral re-activation

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Capable of establishing both productive and latent infections in several lepidopteran cells, *Hz*NV-l virus has been recognized as a model system for studying the mechanism of molecular switching between these two infection phases. In this report, we have identified a highly expressed early gene *hhi1* mapping to the *Hind*III-I fragment of *Hz*NV-l virus during productive infection. In later functional studies, we found that *hhi1* can strongly induce apoptosis through the activation of caspase 3. Further experiments indicated that *hhi1* activates apoptosis through an inhibition of apoptosis 2 (Ac-*iap2*)-inhibitable cysteine protease pathway. Since *hhi1* is one of the very few earliest genes gave rise strong transcripts upon *Hz*NV-l viral infection, possible viral re-activation in latently infected cells by *hhi1* was tested. A significant titer of the re-activated viruses was induced upon transfection of *hhi1* into the latently infected cells, suggesting this gene also plays a critical role for viral re-activation. Latent and productive viral infections are commonly observed phenomena in vertebrates and invertebrates, however, so far very little is known about the switching of viral infections in this important field. Our discovery should provide useful clue for exploring further insights into this intriguing field in the future.

Poster / Virus. Wednesday, 16:30. **V-08**

Functional analysis of two iap genes (iap2 and iap3) of *Lymantria xylina* multiple nucleopolyhedrovirus (LyxyMNPV)

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Baculoviral anti-apoptotic genes p35 and iap (inhibitor of apoptosis) family have been well studied. Baculovirus usually has two or more iap genes, however, not all iaps have anti-apoptotic activity. Two iaps, ly-iap2 and -iap3, from Lymantria xylina multiple nucleopolyhedrovirus (LyxyMNPV) were cloned for functional study. The mRNA expression profiles of these two genes in a permissive cell line, IPLB-LD-652Y (LD cells), were evaluated by quantitative PCR (q-PCR) and RT-PCR. The transcripts of ly-iap2 and -iap3 in the LD cells infected with LyxyMNPV were increased from 6 to 72 hours postinfection (pi), but declined at 3 days to 5 days pi. Interestingly, the transcripts of ly-iap2 and -iap3 in the LyxyMNPV-infected SF21-AE cells showed a significant different phenomenon, the transcript of ly-iap2 was not detected but that of ly-iap3 was detectable, while ly-iap3 in the LyxyMNPV-infected SF cells presented a delayed transcription pattern, it was detected first at 2 days pi and continued to increase at 5 days pi. Functional assay of these two iaps were performed by over-expression method. Full length of LY-IAP3 and BIR domain of LY-IAP2 were needed to inhibit the apoptosis of SF cells which was induced by Drosophila RPR protein. These two iaps are necessary to further evaluate their roles on LyxyMNPV-infected LD and SF cells.

Poster / Virus. Wednesday, 16:30. **V-09**

Functional analysis of the putative antiapoptotic genes, *p49* and *iap4*, of *Spodoptera litura nucleopolyhedrovirus* with RNAi

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A homology search of public database revealsthat *Spodoptera litura* nucleopolyhedrovirus (SpltNPV) possesses two putative, antiapoptotic genes, *p49* and *iap4*; but the function of them has not been investigated in its native host cells. In the present study, we used RNAi to silence the expression of *Splt-iap4* and *Splt-p49*, respectively or synchronously, to determine their roles during the SpltNPV life cycle. RT-PCR analysis and Western blotting showed the target gene expression had been knockdown in the SpltNPV-infected SpLi-221 cells after treated with *Splt-p49* or *Splt-iap4* dsRNA, respectively, confirming that the two genes were effectively silenced. In SpltNPV-infected cells treated with *Splt-p49* dsRNA, apoptosis was observed beginning at 14 h, and almost all cells had undergone apoptosis by 48 h. In contrast, budded virus production and polyhedra formation progressed normally in infected cells treated with *Splt-iap4* dsRNA.Cell viabilitiesanalysis showed that Splt-IAP4has no synergistic effect on inhibition of apoptosis of SpLi-221 cells induced by SpltNPV infection. Interestingly, after *Splt-iap4* dsRNA treatment, cells didn't congregate as those infected with SpltNPV in early infection phase, implying an unknown role of baculovirus *iap4*. Our results determine that *Splt-p49* is necessary to prevent apoptosis; however, *Splt-iap4* has no antiapoptotic function during SpltNPV infection.

Poster / Virus. Wednesday, 16:30. **V-10 STU**

Anterograde trafficking of *Autographa californica* multiple nucleopolyhedrovirus is microtubule-dependent

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Baculoviruses are occluded DNA viruses that are pathogenic to insects. Upon entry into permissive cells baculoviruses are transported into the nucleus via actin polymerisation for virions production. However, it is unclear how baculoviruses exploit the host cytoskeleton for anterograde trafficking. In this study *Autographa californica* Nucleopolyhedrovirus (AcNPV) was used and recombinant baculoviruses: AcEGFP-ORF1629, AcEGFP-VP39 and AcGP64-DsRed that express fluorescently tagged capsid proteins ORF1629 or VP39 or membrane glycoprotein GP64 were constructed. Virus growth curve analysis showed no significant difference between the wild type AcNPV and recombinant baculoviruses. Using confocal scanning microscopy, we found that at 24hpi EGFP-ORF1629 had polarised in a non-continuous pattern in the ring zone of the nuclei of TN368 cells. It is possible that ORF1629 is involved in nuclear budding. At 36hpi, AcEGFP-ORF1629 virion-like structures were found associating with microtubules, which suggests that baculoviruses utilize microtubules for anterograde transport, similar to Vaccinia virus. Cytoplasmic F-actin was found strongly colocalizing with VP39, ORF1629 and GP64 at 48hpi along the cell periphery, possibly indicating cytoplasmic F-actin may be involved in baculovirus budding. We therefore postulate that baculovirus is transported to the cell periphery on microtubules and then through interplay of actin and other unidentified proteins baculovirions bud via plasma membrane acquiring GP64.

Poster / Virus. Wednesday, 16:30. **V-11 STU**

Structural analysis for cypovirus polyhedrin

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Cypoviruses, a member of the family *Reoviridae*, are one group of insect virus that produce micrometer-sized protein crystals called cytoplasmic polyhedra. At the late stage of infection, polyhedra are produced in the cytoplasm of the infected cells and many virus particles are occluded in polyhedra to protect them against extracellular environment. Polyhedra have unique characters, they are very stable against UV, desiccation, any solutions with a wide range of pH (lower than 10), and there is no effect of decomposition by micro organisms. We have determined the atomic structure of *Bombyx mori* cypovirus polyhedrin using single-crystal X-ray diffraction. We found that polyhedra were made of trimers of the 28kDa viral polyhedrin protein. There is a three-fold channel at the centre of the trimers. At the channel, His76 of polyhedrin are located about 10Å by each other. We suggest that it has some important roles to make up the channel. In this study, several mutations are introduced at His76 and the structural changes of wild-type and mutant polyhedrin are analyzed. Polyhedra are considered to be good samples for data collection ofhigh resolution X-ray powder diffraction, the structural analysis of the polyhedrin is conducted by X-ray powder diffraction in SPring-8.

Poster / Virus. Wednesday, 16:30. **V-12**

Identification of viral factors required for the enhancer-like function of baculovirus polyhedrin upstream (*pu*) sequence

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Previously, we identified a novel enhancer-like element, the *polyhedrin* upstream (*pu*) sequence, in the genome of the baculovirus *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV), which activates several early promoters. The activation requires co-infection of AcMNPV, suggesting that viral gene products are needed for *pu*-mediated promoter activation. DNA replication assay showed that the *pu* sequence did not assist in DNA replication and suggested its involvement in activated transcription from target promoters. In order to identify the viral genes responsible for *pu*-dependent activation of early promoters, a set of overlapping cosmid clones covering the entire 134 kb AcMNPV genome were constructed and screened. Our results identified three viral genes *ie1*, *ie2*, and *pe38*, which function in concert with *pu* to activate target promoters. In addition, *pu* and the homologous region (*hr*) of AcMNPV, a known baculovirus enhancer, functioned synergistically, rather additively, to stimulate promoter activity in the presence of these three transactivators.

Poster / Virus. Wednesday, 16:30. **V-13**

Identification of putative miRNA sequences in four insect pathogenic viruses

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MicroRNAs (miRNAs) are a class of small, RNAs found in plants, animals and viruses capable of post-transcriptional regulation of specific mRNAs by inhibiting their translation. A number of different viruses including Herpes viruses and SV 40 have been shown to code for miRNAs. Most viral miRNAs are contained within a ~200 nt primary miRNA transcript which is processed into a ~ 75 nucleotide long pre-miRNA domain capable of forming a stem-loop (hairpin) structure with a calculated minimal folding free energy less than -25kcal/mol. These pre-miRNAs are then cleaved into a ~22 nt long single stranded active miRNA. Here we report on our analysis of the genomes of four insect pathogenic, DNA viruses for the presence of putative miRNA sequences. The salivary gland hypertrophy virus (SGHV) of tsetse flies was found to contain a total of 7 putative miRNAs and the genome of the closely related house fly virus, MdSGHV had 6 putative miRNA sequences. AcMNPV was found to have the fewest miRNAs with only 4 of these sequences present while Hz-2V contains 14 putative miRNAs. The sequence and location of these miRNAs is presented as well as the possible role they might play in the pathology of these viruses.

Poster / Virus. Wednesday, 16:30. **V-14**

MicroRNAs expressed in larval gypsy moth cells post parasitization by *Glyptapanteles flavicoxis* parasitoid

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MicroRNAs (miRNAs) are small noncoding RNAs that regulate gene expression by binding partially complementary sites in mRNAs of targeted genes. Many viruses encode miRNAs that interfere with cellular gene expression. Polydnaviruses (PDVs) are associated with parasitoid wasps and are introduced into host larvae during parasitization, where they infect host cells and cause immune disruption, developmental arrest, and other effects. Several PDV genomes have been recently sequenced, but known miRNA binding sites in PDV genomic sequences have not (yet) been identified by computational methods. To examine host miRNA activity in response to parasitization/PDV infection, gypsy moth, Lymantria dispar, larval hemocytes collected 24h post-parasitization (a time characterized by high levels of PDV and host gene transcription) with parasitoid Glyptapanteles flavicoxis were surveyed for insect miRNAs by microarray hybridization on a Paraflo microfluidic chip. Numerous miRNAs identical to miRNAs from Drosophila and/or other insect genomes were identified with statistical significance in parasitized/GfBV-infected Ld hemocytes. Differential expression of these miRNAs in parasitized vs. non-parasitized larvae was validated using real-time qPCR with miRNA-specific TaqMan assays, which demonstrated greatest abundance of mir-277, -289, and -1 in infected hemocytes. Functional activities have been explored for few miRNAs in Drosophila. Potential roles for expressed cellular miRNAs include anti-viral response.

Poster / Virus. Wednesday, 16:30. **V-15**

Metagenomics of glassy-winged sharpshooter, *Homalodisca vitripennis* (Hemiptera: Cicadellidae)

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A Metagenomics approach was used to identify unknown organisms which live in association with the glassy-winged sharpshooter, *Homalodisca vitripennis* (Hemiptera: Cicadellidae). Metagenomics combines molecular biology and genetics to identify, and characterize genetic material from unique biological samples, these may be environmental, or biological as in animals or plants. The information is then used towards solving a problem. The genetic diversity is assessed by isolation of genetic material (DNA and/or RNA) followed by direct cloning of genes. Three newly discovered single-stranded RNA viruses, along with three bacteria, and one potential fungi were identified. The viruses are undergoing full genome sequencing and provide new taxonomic information to classify these viruses. These viruses may also provide biological control agents for future use against leafhopper pests, or provide gene expression systems for future studies in leafhoppers. The number of sequences returning a top homology match to other species provided matches to *Drosophila melanogaster*, (~12,500) followed by *Aedes aegypti*,*(~9,600), Tribolium casteum*,*(~9,000) Anopheles gambiae*,*(~8500), Nasonia vitripennis*,*(~8,000), Apis mellifera, (6,000)* and then *Homo sapiens* (~4,500). Metagenomics is a new and exciting field of molecular biology that is growing into the standard technique for understanding biological diversity.

Poster / Virus. Wednesday, 16:30. **V-16**

***Malacosoma neustria* nucleopolyhedrovirus (ManeNPV): Replication in Md203 cell line and host range in cell culture**

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The Turkish isolate of *Malacosoma neustria* nucleopolyhedrovirus (ManeNPV) has been characterized based on *in vitro* replication properties in Md203 cell line and host range *in vitro*. Typical cytopathic effects (CPE) of NPV like granulated and rounded cells, nuclear hypertrophy and impairment in cell proliferation, and losted cell shape with the extendings were observed during the infection. We also observed a significant level of viral DNA synthesis by 24 h p.i. Budded viruses were detected at 24 h p.i., but, significent increase in BV production was observed after 48 h p.i. Polyhedral inclusion bodies was firstly detected at 36 h p.i. and the number of inclusion bodies increased by time. The host range of ManeNPV was studied in TniH5, LdElta, BmSPC-36, Sf-21 Se, Cf and Md203 (control) cell lines based on DNA replication and budded virus production. Results showed that ManeNPV replicates in Tni and LdElta cell lines. Inclusion body formation was detected at 36 h and 24 h post-infections for Ld and Tni cells respectively. DNA replication was detected 24 h latter in both cell lines. The yield of budded virus production was 10 fold in both cell lines by 120 h.p.i. The results indicates that The Turkish isolate of ManeNPV has been propogated in cell culture. However, the yield of virus product is needed to be increased in cell culture based system.

Poster / Virus. Wednesday, 16:30. **V-17 STU**

The characteristics and viral susceptibility of the LD cloned cells, IPLB-LD-652Y cell strains-a~f

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The gypsy moth (Lymantria dispar) cell line, IPLB-LD-652Y, was subcloned and six cell strains, IPLB-LD-652Y-a~f, were successfully cloned. These cell strains contain different proportions of four morphological different cells, round, spindle, squamous, and polymorphous cells, the dominant cells in LD-d cells are elongate spindle cells (bipolar or monopolar neuron-like cells), in LD-a cells are polymorphous cells, and in LD-b, -c and -f cells are round cells. These cell strains showed the same esterase isozyme pattern with parent cell line. LD-b and -f cells contained more lipid droplets in cytoplasm than other cell strains, while the lipid droplets were dispersed evenly in LD-b cells and aggregated in LD-f cells. All the cell strains and their parent cells were highly susceptible to LyxyMNPV-5 and LyxyMNPV-G, which were isolated from the moribund larvae of L. xylina with nucleopolyhedrosis, and also fluorescent recombinant viruses, LyxyExp-EGFP and LyxyExp-Red. LD-a and LD-f cells were the highest production of polyhedra and GFP, respectively. All the cell strains were almost 98% susceptible to the viruses. In addition, these cell strains were also susceptible to the RNA viruses from Honeybee, Apis mellifera.

Poster / Virus. Wednesday, 16:30. **V-18**

Applying an *Anticarsia gemmatalis* multiple nucleopolyhedrovirus (*Ag*MNPV)-based direct cloning system to make a cDNA expression library of the cottonwood borer beetle (*Plectrodera scalator*).

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Recombinant baculoviruses are ideal systems for over expressing foreign insect genes in insect cells or whole insects. We have been developing a direct cloning expression vector system in the baculovirus *Ag*MNPV that is suitable for making cDNA expression libraries. Recently, we have been applying this AgMNPV-based cDNA expression library platform to clone expressed genes of the digestive system of the wood boring beetle, Cottonwood borer (*Plectrodera scalator*). Cottonwood borer is a close relative to Asian longhorn beetle (*Anoplophora Glabripennis*), an invasive species to North America. Wood boring beetles cause economically significant damage to forests and there are few beetle control options. Using baculovirus biotechnology to characterize cottonwood borer wood digesting enzymes may lead to new approaches to control wood boring beetle pests and may provide enzymes useful in the paper and the cellulose-based biofuels industries.

Poster / Virus. Wednesday, 16:30. **V-19**

Optimization for high-throughput expression of recombinant protein using EasyBac system

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Recently, we constructed a novel recombinant baculovirus genome, bEasyBac, enabling easy and fast generation of pure recombinant baculovirus without any purification step. In the bEasyBac, bacteriophage lambda site-specific attachment (*att*) sites were introduced to facilitate the generation of recombinant viral genome by *in vitro* transposition. Moreover, extracellular RNase gene from *Bacillus amyloliquefaciens*, barnase, was expressed under the control of *Cotesia plutellae* bracovirus (CpBV) ORF3005 early promoter to negatively select against non-recombinant background. The bEasyBac could replicate in host insect cells only when the barnase gene was replaced to gene of interest by *in vitro* transposition. When the bEasyBac was transposed with pDualBac-EGFP and the EGFP expression efficiency along passage was investigated, the resulting recombinant virus, EasyBac-EGFP, showed comparable level of EGFP expression efficiency with the plaque-purified recombinant virus, AcEGFP, which was constructed using bAcGOZA system, whereas, the non-purified AcEGFP showed quite reduced level of EGFP along passages. Moreover, no non-recombinant backgrounds were detected from unpurified EasyBac-EGFP stocks. Based on these results, high-throughput condition for generation of multiple recombinant viruses in a time was established. These results suggest

that the bEasyBac has an effective benefit enabling for high-throughput baculovirus expression vector without purifying recombinant virus.

Poster / Virus. Wednesday, 16:30. **V-20**

Enhancement of recombinant proteins production in non-lytic insect cells expression system through simultaneously expression of baculovirus encoded transcriptional factor

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We had identified the RhPV IRES (derived from *Rhopalosiphum padi* virus) and PnV539 IRES (derived from *Perina nuda* Picorna-like virus) can functional well in baculovirus infected sf21 cells. In this report, we constructed two bicistronic plasmids, pIB-D-Pn-E and pIB-D-Rh-E, that containing the RhPV IRES or the PnV539 IRES, respectively, and controlled by the *ie2* promoters of Orgyia pseudotsugata multiple nucleopolyhedrovirus (*Op*MNPV). When Sf21 cells transfected with pIB-D-Pn-E and pIB-D-Rh-E, respectively, we found that only the pIB-D-Pn-E transfected cells revealed both the green fluorescence and red fluorescence but the pIB-D-Rh-E transfected cells did not reveal the green fluorescence and only the red fluorescence was observed. Thus, we conclude that the PnV539 IRES but not RhPV IRES can function well in Sf21 cells. Furthermore, we replaced the DsRed genes with the baculovirus encoded transcriptional factor genes, *ie1* and *ie2*, respectively, in pIB-D-Pn-SEFP plasmids. As quantified the medium of both plasmids transfected Sf21 cells, we found that the IE2 rather than IE1 can enhanced the expression of *sefp* gene up to six folds. These results indicated that bicistronic expression vectors can simultaneously express baculovirus IE2 proteins with gene of interest and enhance the recombinant proteins production in a baculovirus-free, nonlytic insect expression system.

Poster / Virus. Wednesday, 16:30. **V-21 STU**

Baculovirus as novel delivery tools for gene therapy in breast cancer

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Autographa californica multinucleopolyhedrovirus (AcMNPV) can efficiently transduce mammalian cell lines with low cytotoxic effects. Breast cancer represents a valuable target for gene therapy and RNA interference (RNAi) has been proposed as an attractive strategy to tackle this disease. Evidence suggests that abnormal glycoproteins in breast cancer cells enhance their invasive potential and baculovirus may offer a novel, efficient and safe alternative as gene therapy vectors carrying RNAi against these molecules. We aim to address whether an AcMNPV-based vector carrying RNAi against *N*-acetylgalactosaminyltransferase-3 (GalNac-T3) is able to reduce the expression of this aberrant glycoprotein in a breast cancer cell line. We transduced MCF-7 cells with a recombinant baculovirus carrying the sequence of a fluorescent protein (DsRed) or RNAi against GalNAcT3. Fluorescent microscopy was used to evaluate the transduction efficiency and qPCR and western blot analysis were done to determine the expression levels of the proteins. Baculovirus carrying DsRed or AcMNPV-siRNA against GalNac-T3 successfully transduced MCF-7 cells with no apparent cytotoxicity and without significant changes in cell viability in comparison with mock cells. Although the expression of GalNac-T3 was still present in the transduced MCF-7 cells, the AcMNPV-siRNA reduced the expression of GalNac-T3 in comparison with the non-transduced cells.

Poster / Virus. Wednesday, 16:30. **V-22**

Molecular cloning and characterization of a glycosyl hydrolase family 9 cellulase expressed throughout the digestive tract of the emma field cricket, *Teleogryllus emma*

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A novel endogenous β-1,4-endoglucanase (EG) gene belonging to the glycosyl hydrolase family (GHF) 9 that is expressed throughout the digestive tract of the emma field cricket, T. emma, was cloned and characterized. This gene consists of eight exons coding for 453 amino acid residues and exists as a single copy in the T. emma genome, named TeEG-I. TeEG-I shares all the features, including signature motifs and catalytic domains, of GHF 9 members, sharing high levels of identity with the termite, Mastotermes darwiniensis (64% protein sequence identity), and the cockroach, Panesthia cribrata (62%), GHF 9 cellulases. Recombinant TeEG-I, which is expressed as a 47-kDa polypeptide in baculovirus-infected insect Sf9 cells, showed the highest activity at 40°C and pH 5.0. Northern and Western blot analyses revealed that TeEG-I was expressed throughout the digestive tract, which correlated with the TeEG-I distribution and cellulase activity in the digestive tract as assayed by immunofluorescence staining and enzyme activity assay, respectively. These results indicate that TeEG-I is expressed throughout the entire digestive tract of T. emma, suggesting a functional role of endogenous TeEG-I in a sequential cellulose digestion process throughout the T. emma digestion tract.

Poster / Virus. Wednesday, 16:30. **V-23**

Obtaining of recombinant human Müllerian Inhibiting Substance (MIS) by using baculovirus expression system

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Mullerian Inhibiting Substance (MIS) is a member of the transforming growth factor b (TGFb) family, a class of molecules that regulates growth, differentiation, and apoptosis in many cell types. In the male embryo, MIS causes regression of the Mullerian duct, the anlage of the Fallopian tubes, uterus, and the upper vagina. Highly purified recombinant human MIS has been shown to inhibit the growth of both human ovarian cancer cell lines and primary tumors *in vitro* and *in vivo*. In our study we have engineered the recombinant baculoviruses, encoded MIS for successful expression of the recombinant human MIS protein that may serve as a new therapy specific to ovarian cancer. The baculovirus expression system has been used widely for expression recombinant proteins encoded by human genes. Conventional baculovirus expression vectors, which were used for the majority of baculovirus-derived recombinant proteins, are recombinant viruses expressing a foreign gene in the insect cells under the control of the *polyhedrin* promoter. As it controls the expression only at the end of baculovirus life cycle, this feature is undesirable for some highly glycosilated foreign proteins because some evidence suggests that cellular glycoprotein processing pathways are compromised at later periods of infection.

In this case, it might be advantageous to use baculovirus vectors that are expressing foreign gene at some earlier stages of the baculovirus life cycle. We have created several recombinant baculoviruses carrying MIS genes under control of early, late and very late baculoviral promoters. Expression cassettes were introduced into several specific loci such that polyhedrin gene retained native. We have compared yield of expression of the foreign MIS gene and identified the optimal promoter for expression of the protein with sufficient yield. Research in the framework of the project was supported by the U.S. Department of State BioIndustry Initiative Program.

Poster / Virus. Wednesday, 16:30. **V-24 STU**

Persistent infection and vertical transmission of *Spodoptera exigua* multiple nucleopolyhedrovirus (Hübner) (Lepidoptera: Noctuidae)

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Vertical transmission is believed to play an important role in the survival of nucleopolyhedroviruses (NPVs) and gives rise, among other effects, to sublethal infections that may influence the severity of insect pest infestations by affecting insect fecundity or fertility. To quantify the prevalence of vertical transmission in *S. exigua* MNPV (SeMNPV) under the greenhouse conditions in southern Spain, wild *S. exigua* adults and their laboratory-reared offspring were screened for SeMNPV by RT-PCR. From a total of 1718 adults captured, 381 females gave rise to offspring. Of these, 6.03% had a persistent infection and 52.17% of the infected females transmitted the virus to their offspring. In contrast, 28.82% of the captured males had a persistent infection. The genotypic variability of the virus isolates collected from cadavers on plants and from the offspring of captured adults which developed an infection, was determined by restriction fragment length polymorphism (RFLP). Six different variants were identified, two of which were most prevalent in progeny larvae. This suggests that such genotypes could preferentially adopt a survival strategy based on vertical transmission.

Poster / Virus. Wednesday, 16:30. **V-25**

Hypermobility and climbing behaviour induced by baculovirus infection are regulated by separate gene functions

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Baculovirus-infected insects show a radical change in behaviour as they become hypermobile and they tend to move up their host plant where they die from virus infection, allowing efficient dissemination of progeny virus over the foliage. This increases the chance of establishing a new round of infection. The central hypothesis is that baculoviruses, besides known genes for virus replication and structure, also contain genes that modulate host behaviour. Deletion of the egt gene from the Group 2 *Lymantria dispar* MNPV resulted in *L. dispar* larvae that did not die at elevated positions, while wild-type infected larvae did. However, deletion of the egt gene from the Group 1 *Autographa californica* MNPV did not alter climbing behavior in either *Spodoptera exigua* or *Trichoplusia ni*, nor did egt-deletion change virus-induced hypermobility. Instead, hypermobility was lost in both hosts by deletion of the Group 1 NPV-specific ptp gene from AcMNPV, but had no effect on peri-mortem climbing behaviour. This climbing behaviour was not affected by light, but was instead an example of negative geotaxis. These results suggest that hypermobility and peri-mortem climbing are distinct behavioural changes induced by two separate viral genes in Group 1 vs. Group 2 baculoviruses, ptp and egt, respectively.

Poster / Virus. Wednesday, 16:30. **V-26**

Comparative pathology of the slow-killing *Adoxophyes honmai* NPV and *Autographa californica* MNPV in *A. honmai*

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Adoxophyes honmai nucleopolyhedrovirus (AdhoNPV) has a distinctive slow-killing pathology compared to most other typical NPVs, such as *Autographa californica* MNPV (AcMNPV). Neonate A. honmai larvae infected with AdhoNPV die after ~17 days and, regardless of the timing of inoculation, infected larvae succumb in the final instar, and do not pupate. To elucidate factors that determine the killing speed of baculoviruses, we compared the general pathology of AdhoNPV with that of AcMNPV, which also infects *A. honmai* larvae but kills them after ~7 days. AdhoNPV showed a similar tissue tropism to AcMNPV, with both viruses infecting fat body and tracheal epidermal cells. However, occlusion body formation in epidermal cells was slower for AdhoNPV than for AcMNPV. The number of occlusion bodies per larva was counted to assess virus production. Growth model parameters were estimated, and revealed that the maximum growth rate was significantly lower and duration of lag phase was significantly longer for AdhoNPV than those for AcMNPV. The gene encoding ecdysteroid UDP-glucosyltransferase (EGT) was transcribed early after inoculation of penultimate-instar larvae with both AdhoNPV and AcMNPV. However, hemolymph EGT activity was detectable only after AdhoNPV-infected larvae molted to the final instar, but could be detected during the penultimate instar in AcMNPV-infected larvae.

Poster / Virus. Wednesday, 16:30. **V-27**

Low oral infectivity of *Ac*MNPV in *Anticarsia gemmatalis* larvae correlates with hemocyte resistance to infection by budded virus.

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Organic soybean is a leading organic crop in the United States that is damaged by infestations of *Anticarsia gemmatalis* larvae. Because baculoviruses are available to organic farmers for controlling crop pests, we characterized infection and pathogenesis of *Autographa californica* M nucleopolyhedrovirus (*Ac*MNPV), in *A. gemmatalis* larvae using an *Ac*MNPV recombinant carrying the *lacZ* reporter gene (*Ac*MNPV-*hsp70lacZ*). Newly-molted fifth instar larvae inoculated orally with occlusion bodies (OB) or intrahemocoelically with budded virus (BV) were highly resistant to fatal infection. Dosages of 5300 OB were required to generate LACZ-positive cells in the midgut, but once infection was established, it was efficiently transferred to adjacent tracheoblasts. However, time course studies revealed that infection did not subsequently disseminate throughout the hemocoel. Lack of distal spread from infected tracheoblasts and low systemic infectivity of BV are suggestive of a virus-elicited immune response, but we did not observe hemocytes associated with LACZ-positive cells. Studies using flow cytometry demonstrate that *A. gemmatalis* hemocytes are resistant to infection by BV, which may explain the low infectivity of *Ac*MNPV. Because neither *Ac*MNPV OB nor BV were highly pathogenic in *A. gemmatalis* larvae, *Ac*MNPV may not be effective in organic cropping systems for controlling *A. gemmatalis* larvae.

Poster / Virus. Wednesday, 16:30. **V-28 STU**

Investigations on the mechanism of CpGV resistance in *Cydia pomonella*

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The *Cydia pomonella* granulovirus (CpGV, Baculoviridae) is the most important biocontrol agent of the codling moth in apple production. In the last 4 years, codling moth populations with an up to 1,000-fold decreased susceptibility to CpGV have been observed in Germany, France, the Netherlands, Italy and Switzerland. The resistance is inherited by a single dominant gene which is located on the Z-Chromosome. A homogeneous resistant codling moth strain was generated by inbreeding a semi-resistant field strain. The LC_{50}s and resistance factors were generated in bioassays with a susceptible, a homogeneous resistant and a semi-resistant codling moth strain. Injections of budded virus into the haemocoel of resistant and susceptible larvae excluded that the CpGV resistance is due to a changed midgut receptor of the virus, and indicated a resistance factor impairing the virus replication and/or virus spread during secondary infection. In order to follow the infection process in susceptible and resistant codling moth larvae, a GpGV-BAcmid expressing the green fluorescent protein (gfp) was constructed.

Poster / Virus. Wednesday, 16:30. **V-29**

Comparison of immune responses in *Cydia pomonella* granulovirus resistant and susceptible strains of *C. pomonella*

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Strains of *Cydia pomonella* (Cp) collected from orchards in Europe have been identified that show resistance to commercial products containing the Mexican strain of *C. pomonella* granulovirus (CpGV-M) as their active ingredient. The virucidal activity and phenoloxidase levels of plasma from two strains of Cp (CpGV-susceptible (CpW and CpSv) and CpGV-resistant (CpRR1 and CpRGV)) were compared. There was a positive correlation between the virucidal activity and phenoloxidase (PO) levels of CpRR1 and CpW and this virucidal activity was inhibited by both PTU and Kojic acid. However, the differential and total haemocyte counts of CpRR1 and CpW were similar. Preliminary data suggests that unlike CpRR1 the high virucidal activity of CpRGV plasma compared to that of CpSv is not related to PO levels in CpRGV plasma.

Poster / Virus. Wednesday, 16:30. **V-30**

Resistance of *Cydia pomonella* to granulovirus: Occurrence in Europe and tests on cross resistance with chemical insecticides

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Codling moth larvae from 23 orchards located in five European countries were tested for their susceptibility/resistance to the Cydia pomonella granulovirus (CpGV) in standardized laboratory bioassays. In general, the results from the bioassays were in accordance with the observations in the field, i.e. most orchards from which the farmer reported failure of the CpGV treatment contained resistant codling moth populations. This was found in all of the countries investigated. The estimation of the percentage of resistant individuals in resistant populations ranged roughly from 30 to 90%. However, in some apparently susceptible populations there were also hints for the presence of a very small fraction of resistant individuals. Fourteen of these European populations were tested for susceptibility to eight insecticides including different classes of insect growth regulators and neurotoxic compounds. High mortality was recorded to most insecticides, ranging from 86% (azinphos-methyl) to 100% (deltamethrin), independent of resistance to CpGV. A reduced susceptibility to azinphos-methyl, chlorpyriphos-ethyl, spinosad and tebufenozide was recorded in several populations. Overall, there was no indication for the occurrence of cross-resistance between CpGV and insecticides in the tested populations.

Poster / Virus. Wednesday, 16:30. **V-31**

Stability of resistance of codling moth against CpGV with and without virus pressure

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Codling moth larvae collected in 2003 in an orchard with severe damage in spite of CpGV treatment proved to be 1000 times less susceptible to the granulovirus (CpGV-M) in bioassays in comparison to a sensitive codling moth laboratory strain. The resistant field strain was reared in the laboratory without virus pressure and maintained a stable 1000-fold resistance level over 32 generations (2.5 years). It was only after this time that the resistance started to decrease gradually. After 10 additional generations (F 42) the larvae were only 10-fold resistant. In additional trials, starting with the 30^{th} generation of the resistant strain, individuals were reared under virus pressure for 5 generations. Already after the first generation, resistance started to increase further. The LC_{50} of the 5^{th} generation after beginning of selection had risen from about $10^{\wedge 6}$ granules per ml diet to $10^{\wedge 8}$ g/ml. This corresponds to a 100,000-fold resistance, which is the maximal resistance to CpGV observed in codling moth.

Poster / Virus. Wednesday, 16:30. **V-32**

Comparative sequence analysis of two entomopoxviruses (EPVs)

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Choristoneura biennis entomopoxvirus (CBEV) and *Choristoneura rosaceana* entomopoxvirus (CREV) have been isolated from the 2-year budworm and from the obliquebanded leafroller, respectively. Both are members of the genus betaentomopoxvirus. *C. biennis* is a conifer defoliating insect in British Columbia, Canada while *C. rosaceana* is a pest of orchard crops and several oranamentals. To date, the genomes of only two EPVs have been fully sequenced, that of *Amsacta moorei* (AMEV) and of *Melanoplus sanguinipes* entomopoxviruses (MSEV). EPV genomes contain A+T residues in excess of 80%, which makes them rather difficult candidates for sequencing. We have attempted conventional as well as the '454' picosequencing to provide an initial overview of the two EPV genomes. The generated sequences were analyzed and compared to those of AMEV and MSEV. The cumulative data showed that even though gene rearrangements were found in CBEV and CREV genomes, gene contents and order were highly conserved among CBEV, CREV and AMEV. The data also indicate that gene content and order may be highly conserved in members of the genus bataentomopoxvirus. Phylogenetic analysis of spheroidin indicates that CBEV, CREV, CFEV and HAEV are very close to AMEV.

Poster / Virus. Wednesday, 16:30. **V-33 STU**

A new entomopoxvirus isolated from tea tortrix, *Homona coffearia*, in Sri Lanka

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The tea tortrix *Homona coffearia* (Lepidoptera: Tortricidae) is one of the most important pests of tea plants in Sri Lanka. In 2005 we found a new entomopoxvirus (EPV) that was infecting approximately 30% of *H. coffearia* larvae collected in up-country tea plantations. We had previously studied an EPV infectious to the oriental tea tortrix *H. magnanima* in Japan, which was initially isolated from *Adoxophyes honmai* (AdhoEPV). We first compared the new Sri Lankan EPV and the Japanese AdhoEPV by analyzing their restriction endonuclease (REN) profiles and pathology in *H. magnanima*. The two EPVs displayed different REN profiles, killing speeds and occlusion body yields, and we therefore designated this new virus HocoEPV. We next asked whether HocoEPV isolates were geographically variable. REN profiles from HocoEPV-infected individuals differed from each other, both within and between plantations. AdhoEPV shows similar variant REN profiles among individuals. In conclusion, EPV host adaptation may be similar for *H. coffearia* populations in Sri Lanka and *H. magnanima* populations in Japan.

Poster / Virus. Wednesday, 16:30. **V-34 STU**

Comparison between two new isolates of PhopGV from *Tecia solanivora* and *Phthorimaea operculella*

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Tecia solanivora is the most limiting potato pest in Colombia, Venezuela and Ecuador where they produce severe damages on stored potato seeds and under field conditions. A Baculovirus, recommended by International Potato Center (IPC), is a good alternative to chemical pesticides, considered dangerous to farmers. To date, only *Phthorimaea operculella* granulovirus (PhopGV), isolated from *P. operculella* infected larvae, were used with variable biological activities. It is important to find specific isolates from *T. solanivora* and to study them at biological and molecular levels. In this work we compare one isolate from *P. operculella* native from La Molina, Peru (Peru6) and one from *T. solanivora* native from Funza, Colombia (Col4). The LD_{50} of Peru6 is 5,881 OBs/mm^2 after multiplication on *P. operculella*, when the LD_{50} of Col4 is 27,38 OBs/mm^2. The analysis of restriction enzyme profiles revealed differences between the 2 isolates. The analysis of 5 variable zones in their genome by PCR using specific primers also revealed differences. The 90-91 gene region revealed a 169 bp deletion in Col4 with a 630 pb band. The biological activity and genetic characteristics are not modified when the isolates are multiplied on their natural hosts.

Poster / Virus. Wednesday, 16:30. **V-35 STU**

Determining the influence of transposon TCl4.7 insertion on the function of the genome of *Cydia pomonella* granulovirus

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CpGV-MCp5 is a natural mutant of the *Cydia pomonella* Granulovirus that harbors an insect host transposon termed TCl4.7 in its genome. TCl4.7 is located between open reading frame Cp15 and Cp16 and separates two hr3 and hr4, which have been recently shown to be origins of replication. As previous competition experiments had demonstrated, that MCp5 has a significant replication disadvantage compared to wild-type CpGV-M. We aimed to study the effect of TCl4.7 insertion on transcription of Cp15 and Cp16 as well as on replication of MCp5. Temporal transcriptional analyses using RT-PCR and quantitative real-time PCR revealed that both Cp15 and Cp16 transcription could be early detected in both CpGV and CpGV-MCp5 infect larvae. However, a significant decrease of Cp15 and Cp16 transcripts could be observed in MCp5. When Cp15 and Cp16 deletion mutants of CpGV were generated using BACmid technology, the resulting CpGV *Cp15*-null BACmid was not able to produce virus infection after injection into *C. pomonella* larvae. In contrast, the generated *Cp16*-null BACmid caused infection and thus was not required for in-vivo infection. In addition, two mutant BACmids with a deletion of hr3 and hr4 and an insertion of a kanamycin cassette in between both hrs were generated to study the function of these hrs and their mimic. Both mutant BACmids could to replicate and produce infectious viruses. Thus, the Cp15 gene is an essential for viral infection cycle but Cp16 gene is not. On the other hand, the interruption of hr3 and hr4 did not affect the viral infection cycle.

Poster / Virus. Wednesday, 16:30. **V-36**

Quantitative PCR analysis of the tsetse fly salivary gland hypertrophy virus (SGHV) in a laboratory colony of *Glossina pallidipes*

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The *Glossina pallidipes* salivary gland hypertrophyvirus (GpSGHV) causes hypertrophy at a low (1-5 %) frequency in natural populations but affects 10% of our laboratory colony of *G pallidipes* and significantly reduces the fecundity of symptomatic flies. To analyze how this virus persists in the colony and to try to correlate hypertrophy syndrome with virus loads, we optimized a quantitative PCR (QPCR) method by designing specific primers in the GpSGHV005 coding sequence (1). The virus loads in asymptomatic flies for excised leg, salivary glands and total fly body averaged 5.58E+5, 7.41E+5 and 7.43E+7 virus copy number (VCN), respectively, whereas in symptomatic flies, virus loads averaged 2.33E+7, 2.15E+10 and 3.53E+10 VCN for the same tissues. Despite these differences, only a slight increase in virus loads was observed in randomly sampled flies from different ages. A clear correlation between virus loads in pupae and their mothers was observed. Taken together, these results 1) confirm a close relationship between virus loads and SGH syndrome in adults and correlate the vertical transmission of GpSGHV from mother to progeny, 2) strongly supports the correlation between the development of SGH in progeny and the virus load acquired by the larva during its intra uterine development. (1) Abd-Alla *et al*. *J. Virol* 2008

Poster / Virus. Wednesday, 16:30. **V-37**

On the wings of Real Time: Detection, quantification, and effects of DWV

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Deformed wing virus (DWV) can persist undetected in honeybees *Apis mellifera*, causing no apparent symptoms. It is known to be associated with the parasitic mite, *Varroa destructor*, and once present can also be transmitted between bees in a colony. Pupae that are heavily infected with DWV will develop as adults with disfigured wings, appendages, and abdomen, possible paralysis, and a highly reduced life-span. The combined weakening effect of the parasite *V. destructor* and DWV is known to potentially result in the death of the colony within three to five years. A methodology is presented for the accurate detection and quantification of DWV in honeybee colonies using Real-Time Reverse Transcription PCR. Firstly, we demonstrate that DWV is present in every individual bee in an infected colony, at varying levels, and decipher the optimal template concentration required for DWV detection in single bees. Secondly, we evaluate the total number of bees necessary to screen, to represent the average level of infection within a colony. Finally, we compare concentrations of the house-keeping gene Actin, the stress protein, heat shock protein 70, and DWV viral load between symptomatic, asymptomatic, and uninfected adults.

MICROBIAL CONTROL WORKSHOP Wednesday, 19:30-21:30

Biological Solutions to Pest Control

Workshop paper. Wednesday, 19:30. **173**

Challenges in commercialization of micro- and macro-biologicals

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Becker Underwood produce and market a number of micro- and macro-biological control products. Our micro-biological products are based on entomopathogenic fungi (EPF) and beneficial bacteria. Our 13 macro-biological products contain entomopathogenic nematodes (EPN) and are manufactured in the worlds largest nematode production facility in Littlehampton, UK. Green Guard (*Metarhizium anisopliae*), a micro-biological product and is sold throughout Australia for the control of locusts and grasshoppers. Nemaslug (*Phasmarhabditis hermaphrodita*) is a macro-biological, this species of nematode was originally isolated from Rothamsted research station, UK and is now widely used throughout Europe to control slugs. This paper will detail how these products have gone from discovery, through laboratory screening and to successful commercialisation. Selling products into over 65 countries, Becker Underwood faces a variety of different registration requirements. Registration requirements are arguably the greatest challenge to companies for the commercialisation of biological control products. Other important issues including mass production, quality control, shipping and applicator training will also be discussed as these can be more demanding than chemical-based plant protection products.

Workshop paper. Wednesday, 19:45. **174**

Development of microbial biopesticides based on entomopathogenic fungi: Research to commercialization

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Large collections of entomopathogenic fungi are maintained throughout the world for research and development purposes. There is a wealth of literature describing the pathogenicity, environmental stress tolerance, and other characteristics of strains from these collections. However, there have been relatively few success stories for strains that have become commercially viable products. Making the leap from a promising isolate to a commercial product requires careful forethought. Some properties of the isolate's to consider are its pathogenicity, host range, environmental compatibility, and compatibility with mass production. Production scale-up and formulation then provide sufficient material for honest field evaluations. Overcoming such technical hurdles are not sufficient for commercial success as the cumulative market niches must provide sufficient return on investments made for technical development and regulatory costs. These themes are common to the commercialization of all microbial biopesticides. However, by exploiting some of the unique benefits of entomopathogenic fungi rather than viewing them as a direct replacement for chemical insecticides, novel niches in IPM can be exploited. Examples will be come from the development of entomopathogenic fungi for areawide management of cotton insects pests and commercial development of products based on the strain Metarhizium anisopliae strain F52.

Workshop paper. Wednesday, 20:00. **175**

Field performance of novel stacked Bt products for protection against corn insects

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HERCULEX® XTRA *Insect Protection* from Dow AgroSciences represents a new generation of transgenic insect resistance traits for maize. HERCULEX® XTRA is a genetic stack of HERCULEX® I *Insect Protection* (*Bacillus thuringiensis (Bt)* Cry1F, event TC1507) and HERCULEX® RW *Insect Protection* (*Bt* Cry34Ab1 and Cry35Ab1, event DAS-59122-7). The Cry1F component of HERCULEX® XTRA protects against larvae of several economically important above ground pests of maize including stalk borers (*Ostrinia nubilalis, Diatraea grandiosella, Diatraea saccharalis, Elasmopalpus lignosellus, Diatraea crambidoides)*, cutworms (*Agrotis ipsilon, Striacosta albicosta*) and fall armyworm (*Spodoptera frugiperda*). The Cry34Ab1/Cry35Ab1 component of HERCULEX® XTRA protects against the larval stages of corn rootworms (*Diabrotica virgifera virgifera , Diabrotica barberi* and *Diabrotica virgifera zeae*). This presentation summarizes Dow AgroSciences' recent field research results for HERCULEX® XTRA that demonstrate broad spectrum protection against above ground and below ground corn pests. Evidence that stacked insect resistance traits contribute to improved agronomic performance will be presented. Last, we describe SmartStax, an eight-way gene stack for enhanced insect spectrum and durability that also incorporates multiple modes of action for weed control.

Workshop paper. Cancelled. **176**

Workshop paper. Wednesday, 20:15. **177**

Development of and prospects for the BtBooster platform technology

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Previously we reported that an enhancer peptide derived from a *Bacillus thuringiensis* (Bt) receptor (BT-R1) could enhance the activity of purified Cry toxins in diet-based bioassays and that a protease-stabilized derivative of this enhancer peptide (called BtBooster™) also enhances the activity of a commercial Bt sprayable product in plant-based bioassays. Here, we report that both wild type and protease-stabilized BtBooster variants have similar dose response curves for enhancement of Cry1Ac when tested against *Helicoverpa zea* larvae in diet surface-treatment bioassays at low Bt to BtBooster mass ratios. However, at higher BtBooster ratios the level of enhancement for the wild type BtBooster declined while the level of enhancement of the modified BtBooster increased further. Field trials with the modified BtBooster are currently in progress. BtBooster alone has proven non-toxic to all insect species tested, and has no homology to known toxins and allergens. BtBooster has significant potential as an enhancing agent for Bt proteins produced in biopesticides and plants.

Workshop paper. Wednesday, 20:30. **178**

RNAi and Bt protein approaches to corn rootworm control

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Currently, all commercialized transgenic approaches for managing western corn rootworm (WCR), *Diabrotica virgifera virgifera* LeConte, and northern corn rootworm, *Diabrotica barberi* Smith and Lawrence, in the USA utilize various Bt proteins as the insecticidal mode of action. While this approach has proven to be an excellent tactic for maize producers in the USA, additional modes of action will likely be required to ensure the long term success of transgenic approaches for rootworm management. We recently reported that ingestion of dsRNAs from selected target gene templates can induce larval stunting and mortality in artificial feeding assays. Furthermore, we demonstrated that maize plants expressing selected dsRNAs protect roots from rootworm feeding damage. Here, we demonstrate that *in-planta* dsRNAs alone and in combination with Bt significantly effect multiple aspects of WCR life history while challenging these pests to a novel mode of action. Beyond root feeding protection, our results indicate that this approach may serve as an effective resistance management option as we move forward with additional options for managing rootworms of maize.

Workshop paper. Wednesday, 20:45. **179**

***Bacillus thuringiensis* - based products: Forever young**

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Advancements in production efficiency, formulation stability, quality control, field efficacy and application strategies of Bacillus thuringiensis (Bt) contributed for their global widely usage in the IPM programs of a wide range of crops, including vegetables, tree fruits and vines. The market for Bt products is approximately US$160 million, which represent approximately 60% of the total microbial insecticides and 1.5% of the total crop protection insecticides market. The annual growth rate of microbial insecticides from 1980 to 2004 has been ranging from 8.0% to 10.0% and is projected to be 10.0% in 2009. For the same period, the annual growth of chemical pesticides has been ranging from – 2.3% to 2.2% and is projected to be 0.75% in 2009. The market potential for Bt products will likely increase in the next few years due to the following: The demand of the regulatory agencies and the general public for safer produce and for products with no detrimental effect to the environment. High probabilities of insect resistance developing against reduced-risk insecticides. An increase in the awareness of the growers, distributors and farm advisors about the benefits and the flexibility of including Bt products in insect management programs. The producers of Bt products are positioning their products as partners and not as alternatives to chemical insecticides.

THURSDAY - 7 August

SYMPOSIUM (Bacteria Division) Thursday 8:00-10:00

Commercialization and Quality Control of Bacterial Insecticides

Symposium. Thursday, 8:00. **180**

Bt standards and the importance of quality control of Bt products

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The value in having Bt products with consistent performance and confirmed safety is an increase in consumer confidence, ultimately leading to increased use and demand of Bt products and potentially other biological control agents. However, poor quality control in even one company can damage the reputation of Bt's and microbials in general. Assuring biopotency of products by using reliable standards is important, but is only one aspect of QC. At Valent BioSciences, quality control measures are applied at all stages of manufacturing, from strain identity to packaging and distribution of the final product. Recently, Bt products have been appearing in the worldwide market that demonstrate an obvious lack of quality control, even though biopotency may be met. In some cases the products have been misrepresented or adulterated. Thus, in addition to implementing high standards of quality control, it is in the interest of the entire biopesticide industry to provide stewardship for all products on the market.

Symposium. Thursday, 8:30. **181**

Bacterial insecticides, commercial development and quality control

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The development of bacterial insectcides has experienced up and down cycles. During the past several years, there have been significant changes in the companies which engage in the development and commercialization of bacteria insecides. Commercialization of products other than Bacillus thuringiensis (Bt) have limited success. Bt based formulations remain the main products in this field Other bacteria failed in the market place due to (1) production issues, (2) market size, (3) efficacy, (3) competition, (4) stability and (5) registration costs etc. Quality control of bacterial insecticides includes the passage of multiple physical properties and the assurance of insect killing power or potency of a formulation. The potency of Bt based insecticides is generally estimated by bioassy, i.e. the measuement of the dose rsponse of target insect to a formulation in comparison to a recognized standard of know potency. There are successes and issues of using alternative methods other than bioassay to determine potency. The limitations of using the alternative methods will be presented and discussed.

Symposium. Thursday, 9:00. **182**

Impact of regulations on commercialization of bacterial insecticides

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The market for products based on biological control agents (BcAs) has been increasing steadily over the past few years at a much faster rate than that of chemical plant protection products. This market will continue to increase in the future as a result of several factors: growth of the niche market of high value crops, withdrawal and/or restrictions in the use of chemical pesticides, ecotoxicological issues, restrictions concerning minimum detectable residue levels in the final produce, consumer awareness, and consequently increased adoption of IPM and sustainable farming strategies. Unfortunately, up to now, the registration process has been completed successfully for only a limited number of BcAs, and for an even lower number full commercial development has been achieved. Several factors, especially regulatory constraints, can negatively affect the successful development and commercialization of cost-effective BcA-based products. A detailed analysis of the different factors involved will be presented.

Symposium. Thursday, 9:30. **183**

Proposals for a balanced regulation of micobial biocontrol agents - results of the REBECA Action

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Within the last two years the Policy Support Action REBECA reviewed currently existing regulation of microbial biocontrol agents and made proposals for improved procedures to accelerate registration and reduce costs while maintaining the high level of safety for users and consumers. In general, REBECA recommended to introduce or maintain the practice of presubmission meetings to define which data are required. Apart from many general proposals for improvement and acceleration of the registration process the Action also provided recommendations specific to microbial biocontrol agents and viruses. The high similarity between baculoviruses justifies a general assessment at the level of the family *Baculoviridae.*For products containing bacteria and fungi, the action defined a short list of data, which should be available for a pre-submission meeting in order to be able to decide on data requirements and waivers. Major concerns exist about how to handle the risk assessment of microbial metabolites. These substances usually have a very short half-life and are generally produced in small quantifies, why many participants of the Action did not consider metabolites of microbials used in biocontrol to pose riks to humans and the environment. A short trak decision pathway was developed for risk assessment of metabolites. Data requirements on effects on earthworms and soil microbiota should be generally waived because hazards are very unlikely. Infectivity studies should be waived when all of the following requirements are met: no clinical reports, not listed in 2001/54 EC, humans and animals are already regularly exposed to the micro-organism, susceptibility to antibiotics. Data requirements regarding the instability of genetic traits affecting the efficacy of the product should be waived or removed because this will be checked by quality control. Data requirements on fate and behaviour in the environment should be waived for micro-organisms which are already part of the background population.

SYMPOSIUM (Virus Division) Thursday 8:00-10:00

Comparative Genomics of DNA Viruses

Symposium. Thursday, 8:00. **184**

Evidence for extensive lateral acquisition of cellular genes by nucleocytoplasmic large DNA viruses

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Nucleo-Cytoplasmic Large DNA viruses (NCLDV), a diverse group that infects a wide range of eukaryotic hosts, exhibit a large heterogeneity in genome size (between 100kb and 1.2Mb) but have been suggested to form a monophyletic group on the basis of a small subset of approximately 30 conserved genes. We demonstrate that many NCLDV lineages appear to have undergone frequent gene exchange in two different ways. Viruses which infect protists directly (Mimivirus) or algae which exist as intracellular protist symbionts (Phycodnaviruses) acquire genes from a bacterial source. Metazoan viruses such as the Poxviruses show a predominant acquisition of host genes. In both cases, the laterally acquired genes show a strong tendency to be positioned at the tip of the genome. Surprisingly, several core genes believed to be ancestral in the family appear to have undergone lateral gene transfers, suggesting that the NCLDV ancestor might have had a smaller genome than previously believed. Moreover, our data show that the larger the genome, the higher is the number of laterally acquired genes. We propose that the NCLDV viruses have evolved by significant growth of a simple DNA virus by gene acquisition from cellular sources.

Symposium. Thursday, 8:24. **185**

Mimivirus and Mimiviridae: Toward a new family of large DNA viruses

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The discovery of Mimivirus (for "Mimicking Microbe" virus)(in 2001), a double-stranded DNA virus infecting common amoeba of the *Acanthamoeba* genus, followed by the analysis of its complete genome (in 2003) sent a shock wave through the community of virologists and evolutionists. By its record particle size (750 nm in diameter – see below) and genome length (1.2 million bp), the complexity of its gene repertoire (911 protein coding genes) as well as of its particle (made of the products of more than 130 virus genes), Mimivirus blurred the established boundaries between viruses and parasitic cellular organisms. As more researchers are getting involved in the study of Mimivirus, experimental information is now slowly accumulating, although very little is yet known on its physiology. I will review some of the recent progresses, including individual protein characterizations, electron microscopy, proteomics, new evidence about the ancestral origin of the Mimivirus lineage, as well as a spectacular, albeit mysterious, example of horizontal gene transfer. Our analysis of recent metagenomic data demonstrates that Mimiviridae are well represented in the sea, and strongly suggests that the closest marine mimivirus relatives are large viruses infecting algae.

Symposium. Thursday, 8:48. **186**

Structural divergence among genomes of closely related baculoviruses and its implications for baculovirus evolution

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Of the 42 baculovirus genomes that have been sequenced to date, some are from closely related viruses that share overall nucleotide sequence identities in excess of 95%. Comparative analysis of genomic sequences from closely related viruses can provide insight into how baculoviruses evolve and adapt to new host species or overcome the defenses of their current hosts. This presentation will focus on the comparative analysis of partial and complete nucleotide sequences from two groups of closely related baculoviruses: (a) multiple nucleopolyhedroviruses (MNPVs) from a cluster including *Autographa californica* (Ac)MNPV, *Rachiplusia ou* (Ro)MNPV, and *Plutella xylostella* (Plxy)MNPV; and (b) granuloviruses (GVs) from a cluster including *Xestia c-nigrum* (Xecn)GV and *Helicoverpa armigera* (Hear)GV. Even though the individual viruses in these clusters have diverged from each other relatively recently, a considerable degree of genomic rearrangement (in the form of insertions, deletions, and recombination resulting in allelic replacement) is evident from alignments of their genomes. Implications for the processes shaping baculovirus genomes and the evolution of biological characteristics of the viruses in these groups will be discussed.

Symposium. Thursday, 9:12. **187**

The genome of *Oryctes rhinoceros* nudivirus: A missing link that solves some mysteries of invertebrate virus evolution

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The *Oryctes rhinoceros* nudivirus (OrNV) contains enveloped, rod-shaped and dsDNA virions, and replicates in the nuclei of infected midgut and fat body cells. The relationship of the nudiviruses to each other, to the baculoviruses as well as to other large dsDNA viruses, including the Monodon baculovirus (MBV), the salivary gland hypertrophy viruses (SGHVs) and white spot syndrome virus (WSSV), is elucidated with the complete genome sequence of OrNV, which is 127,615 bp in size with an AT content of 58% and contains 139 predicted protein-coding open reading frames (ORFs). In-depth genome sequence comparisons revealed that the nudiviruses share 20 baculovirus core gene homologues associated with transcription (*p47*, *lef-8*, *lef-9*, *lef-4*, *vlf-1*, and *lef-5*), replication (*dnapol* and *helicase*), virus structure (*p74*, *pif-1*, *pif-2*, *pif-3*, *vp91*, *vp39*, *38K*, *19kda*, and *odv-e56*), and unknown functions (*ac68*, *ac81*,and *p33*). Four of these conserved genes are present in the partially sequenced MBV; eight are present in the SGHVs. Homologues of the four *pif* genes (*p74*, *pif-1*, *pif-2* and *pif-3*) of baculoviruses were also identified in the nudiviruses, the SGHVs, and surprisingly in WSSV. In baculoviruses, these *pif*s are involved in virus binding and entry into midgut epithelial cells and hence are essential for successful infection of insect hosts *per os*. It is now assumed that their mode of action is highly conserved in the arthropods including crustaceans. Based on phylogenetic analyses of DNA polymerase and the PIFs, we propose that WSSV and the SGHV diverged early from a common ancestor of the nudiviruses and the baculoviruses. Genome wide analysis indicate that these invertebrate-specific circular dsDNA viruses are more closely related to each other than to any other large eukaryotic dsDNA viruses sequenced so far.

Symposium. Thursday, 9:36. **188**

Wasp-bracovirus associations: The grail quest for the ancestor virus

Annie Bézier[1]; Marc Annaheim[2]; Juline Herbinière[1]; Christoph Wetterwald[3]; Gabor Gyapay[4]; Sylvie Bernard-Samain[4]; Patrick Wincker[4]; Isabel Roditi[2]; Manfred Heller[2]; Maya Belghazi[5]; Jérôme Lesobre[1]; Rita Pfister-Wilhem[2]; Georges Periquet[1]; Catherine Dupuy[1]; Elisabeth Huguet[1]; Nathalie Volkoff[6]; Beatrice Lanzrein[2]; Jean-Michel Drezen[1]
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Comparative genomic studies have highlighted the role of symbiotic associations in biological evolution. However very few of these relationships involve viruses, except the remarkable association of polydnaviruses (PDVs) with tens of thousand species of parasitic wasps that develop within the body of lepidopteran larvae. PDV particles, injected along with parasite eggs into the host body, act by manipulating host immune defences, development and physiology thereby enabling wasp larvae to survive in a potentially harmful environment. The virus is completely dependent on the wasp for particles production that occurs exclusively in specialized cells of the ovaries. Surprisingly, the genome enclosed in the particles encodes almost no viral structural protein but mostly factors used to manipulate the parasitized host. It was thus questioned whether PDVs were true viruses or a genetic secretion somehow created by the wasp. We unravelled recently the viral nature of PDVs associated with braconid wasps by characterizing a large set of virus genes encoding structural components of PDV particles in the braconid species *Chelonus inanitus* and *Cotesia congregata* which belong to the most distantly related subfamilies of bracovirus-associated wasps.

CONTRIBUTED PAPERS (Cross-Divisional) Thursday, 8:00-9:30

Pathogens of Bees

Contributed paper. Thursday, 8:00. **189**

A sticky situation: Picorna-like viruses infecting U.K. honeybee populations

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Screening of honeybee colonies located in Devon, South West England for 6 picorna-like viruses revealed a pool of high genetic diversity within different isolates of DWV and ABPV. Studies of the RNA-dependent RNA polymerase highlighted it's usefulness as a marker for studying these viruses and supported theories that DWV, VDV and KV are variants of the same virus, as well as potentially ABPV, KBV and IAPV. This information was used when designing primers for real-time PCR analysis of viral occurrence in honeybee colonies. The amount of total RNA required for quantifying DWV viral load was investigated, along with the total number of honeybees needed to be screened to provide an adequate representation of the level of infection within a colony. Honeybee samples were then collected from 3 colonies within an apiary located in South West England over the course of an annual cycle, with 2 of the 3 colonies surviving and 1 colony suffering a collapse at the end of the sample period. Quantitative PCR was used to investigate the occurrence of DWV, ABPV, BQCV and SBV during this time. Observations on the virus occurrence and load within the colonies will be presented.

Contributed paper. Thursday, 8:15. **190 STU**

Deformed wing virus in the parasitic mite, *Tropilaelaps* spp.

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Mites in the genus *Tropilaelaps* (Acari:Laelapidae) are parasites of the brood of honey bees (*Apis* spp.). *Tropilaelaps clareae* is described from *Apis dorsata*, but the mite also parasitizes the European honey bee, *Apis mellifera*. Infestations can rapidly lead to the death of entire bee colonies and *T. clareae* is hence considered more dangerous to European bees than the parasitic mite *Varroa destructor*. Honey bees are infected by many different viruses, some of them associated with and vectored by *V. destructor*. The most prevalent virus infection in honey bees in recent years, associated with *V. destructor* appears to be deformed wing virus (DWV). DWV is distributed world-wide, and found wherever the Varroa mite is found. The Varroa mite transmits viral particles when feeding on the haemolymph of pupae or adult bees. Both the *Tropilaelaps* mite and the *Varroa* mite feed on honey bee brood, but no observations of DWV in *Tropilaelaps* have so far been reported. In this study, we used a novel quantitative real-time RT-PCR to investigate the occurrence of DWV in infested brood and *Tropilaelaps* mites collected in China. We can, for the first time, report occurrence of DWV in *T. clareae* and demonstrate a close association between mite-infested pupae of *A. mellifera* and DWV infections.

Contributed paper. Thursday, 8:30. **191 STU**

Honeybee immunity and parasitism by *Nosema* spp. fungi and *Varroa* mites

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Nosema apis, *N. ceranae*, and *Varroa destructor* are particularly detrimental to honeybee (*Apis mellifera*) colony productivity and survival. We are measuring honeybee immune responses to infection by each fungal (microsporidian) species alone and in combination with mites. We are also measuring effects of chemotherapy on honeybee immunity. Quantifying these trade-offs through biochemical analysis of immune proteins may enable us to determine infection threshold levels for effective use of chemical treatments, thereby reducing the risk of *Varroa* or *Nosema* evolving chemical resistance. Finally, we are testing if immune protein concentrations resulting from parasitic infection predict honeybee survival, potentially leading to a means of assessing mortality risk in advance of over-wintering of honeybee colonies.

Contributed paper. Thursday, 8:45. **192 STU**

Does fumagillin control the microsporidian *Nosema ceranae* in western honey bees (*Apis mellifera*)?

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Nosemosis in western honey bees (*Apis mellifera*) is caused by the microsporidians *Nosema apis* and *N. ceranae*. Pathology associated with *N. apis*, the historical parasite of western honey bees, is well understood, and includes increased winter mortality and poor spring build-up of surviving colonies. Conversely, pathology associated with recently-detected *N. ceranae*, historically of Asian honey bees (*Apis cerana*), is not well-described. *N. ceranae* was associated with increased winter mortality and reduced honey yields in Spain, and was highly pathogenic when inoculated experimentally. The antibiotic fumagillin dicyclohexylammonium (hereafter, fumagillin) is used to control *N. apis*; however, it is unclear whether fumagillin is effective against *N. ceranae*. To determine this, western honey bee colonies in Nova Scotia, Canada were sampled in spring and late summer 2007. *Nosema* intensity in the spring was significantly lower in colonies treated with fumagillin in September 2006 (n = 94) than those not treated (n = 51), but by late summer no difference existed between groups. Molecular sequencing of 15 infected colonies identified *N. ceranae* in 93.3% of cases, suggesting that fumagillin is successful at temporarily reducing this recent invasive parasite in western honey bees.

Contributed paper. Thursday, 9:00. **193 STU**

Environmental effects on fungal infections in honeybee larvae *Apis mellifera* (Hymenoptera: Apidae)

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Among the social insects, honeybees *Apis mellifera* have an exceptionally diverse set of parasites and pathogens. In this study two species of fungal diseases have been investigated: one is the common brood diseases, chalkbrood (*Ascosphaera apis*) and another opportunistic, but less common pathogen in honeybees, the stonebrood (*Aspergillus flavus*). Using the honeybee larvae as host and these two pathogens we investigated *in vitro* temperature impacts on the infected larvae. Temperature is known to have a crucial role in mediating the outcome of the host – parasite interactions; however there is limited information on the possible competition among fungal pathogens within the honeybee host. In addition, we investigated within-host competition among different fungal pathogens within a single larva and the role temperature plays in mediating these interactions.

Contributed paper. Thursday, 9:15. **194**

Asexual reproduction in the honey bee fungal pathogen *Ascosphaera apis*

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Ascosphaera apis is an important fungal pathogen of honey bees. *A. apis* produces sexual spores (ascospores) that are the primary infective agent of chalkbrood disease. Honey bee larvae can be infected with *A. apis* by ingesting larval food contaminated with ascospores. By contrast, asexual reproduction has never been described in *A. apis*, although it is a widespread form of propagation in Ascomycetes. Since asexual reproduction does not require mating, it allows rapid production of large numbers of conidia (mitospores), and their subsequent dispersal into new areas. This study thus fills an important gap in current understanding of the developmental cycle of an important fungal honey bee pathogen. Herein we describe asexual reproduction in *A. apis* and discuss its potential role in host pathogenesis and in the dissemination of this infectious bee disease in the environment. Considering the worldwide spread of chalkbrood disease and the lack of EPA approved drugs to cure it, an understanding of the *A. apis* life cycle is an important factor in the design of a disease management program.

SYMPOSIUM (Cross-Divisional) Thursday, 14:00-16:00

Role of Disease in Regulation of Non-Pest Populations

Symposium. Thursday, 14:00. **195**

Specialist and generalist entomopathogenic fungi infecting non-pest insects: Implications for ecosystem services and relevance of behavioural ecology

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Entomopathogenic fungi infect a wide array of insects from most orders and they are among the natural enemies that contribute to the regulation of insect populations. However, only a limited number of studies have focused on the impact of fungal pathogens on populations of non-pest insects. Effects of entomopathogenic fungi on non-pest host populations should receive more attention based on the increasing interest in conservation biological control. In this strategy, founded on competition theory, non-pest host populations adjacent to cropping systems will in principle affect pest populations through shared natural enemies. We present examples of selected non-pest host-fungus systems from temperate ecosystems that are relevant for the expected ecosystem service provided by entomopathogenic fungi. Predators are among the non-pest hosts that are infected by fungi. Recent advances in our understanding of the effect of pathogens on the behaviour of predators may shed light on the significance of entomopathogenic fungi for the regulation of predator populations. We discuss what we can learn about host-pathogen interactions from behavioural ecology and which life history parameters in the host that may be important for the impacts of fungal pathogens on their host populations.

Symposium. Thursday, 14:24. **196**

Covert viruses in wild populations

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Lepidoptera are attacked by numerous virus strains, but in many cases do not show obvious signs of infection. Molecular techniques now allow the monitoring of viruses in wild populations without overt disease, and this has revealed a surprising diversity of pathogens. Baculoviruses were traditionally known for their lethal impact on hosts but are now known to also form persistent, almost symptomless infections, first detected in *Mamestra brassicae*. Such hidden infections may be vertically transmitted over many generations, be vectored by pathogens, have major to minimal impacts on host fitness and may interact with other invading pathogens. We report the detection of covert infections caused by baculoviruses and cypoviruses in a range of species, and explore their ecological significance.

Symposium. Thursday, 14:48. **197**

Microsporidian disease in beneficial insects

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Entomopathogenic microsporidia produce chronic infections that often do not produce obvious symptoms.This group of primary pathogens is, therefore, best known in managed insects or in well-studied pest populations. Microsporidiosis of domesticated insects such as honey bees and silkworms are known to cause serious effects on colony health and productivity. In the field situation, however, microsporidian disease is more difficult to observe and the effects on non-pest wild insects have rarely been studied. Nosema bombi, a microsporidian pathogen of bumble bees (Bombus spp.) was implicated in the decimation of commercially produced Bombus occidentalis in the early 1990's in California, but the effects of this pathogen on natural Bombus populations has only recently been addressed. Other issues involve the use of exotic insects in classical biological control programs that may be infected with microsporidia. This presentation will address both the current situation concerning microsporidiosis in Bombus spp. in North America, and that of a microsporidium infecting a coleopteran predator, Sasajischymnus tsugae, of the hemlock woolly adelgid, Adelges tsugae.

Symposium. Thursday, 15:12. **198**

Methods for studying pathogens in natural populations: Recent developments and future thoughts

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Most studies on insect pathogens are within the context of insect pest control and there has, in comparison, been little research into the role that pathogens may play in regulating natural populations of insects. Studies of pathogens in natural populations present a number of methodological and sampling challenges. For example, the host range of a pathogen within a natural insect population may be difficult to define as groups of unrelated hosts may be infected. In comparison to agroecosystems there are generally a greater number of species in natural habitats making it necessary to precisely define the particular habitat a host may occupy. Host density may also be low and therefore pathogen epizootics may not occur regularly making direct observations of pathogens difficult. Sampling the habitat in these cases may be more useful in assessing the prevalence of particular pathogen groups. Sampling strategies also need to account for host phenology as pathogens may occur as low level, covert infections present in different host life stages and at different frequencies during host development. I refer to examples of methods being used in a project to assess the prevalence and distribution of UK Lepidoptera pathogens and draw on work from other research groups.

Symposium. Thursday, 15:36. **199**

Parasites mediate biological invasions

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Parasites can affect the outcome of biological invasions in different ways. Outbreaks of parasites may lead to host population crashes and resultant community change. But parasites do not only act on host population density. We present studies of short-term, behavioural effects of parasites and their effect on invasions. We focus on parasite regulation of crustacean invasions. Using empirical studies and mathematical modelling, we show that two parasites play keystone roles in UK amphipod invasions. Firstly, the microsporidian *Pleistophora mulleri* may facilitate invasion by two smaller species of amphipod*;* it has no direct effect on the survival of the native *G. d. celticus*, but infected animals are less likely to prey on the two smaller invaders. Secondly, the acanthocephalan *Echinorynchus truttae* may promote coexistence, as infection of the invading species *Gammarus pulex* reduces its predation on native *G. d. celticus.* Microsporidia may also drive crayfish invasions. We provide evidence from sequence data that the invading signal crayfish has acquired *Thelohania contejeani* (porcelain disease) from the native. However, whilst the invader may suffer little from the infection, transmission to the native can cause reduced activity and mortality and so increase the rate of extinction of this species.

CONTRIBUTED PAPERS Thursday, 14:00-15:30

BACTERIA 4

Contributed paper. Thursday, 14:00. **200**

Genetic improvement of the Cry11 from *Bacillus thuringiensis* subsp. *medellin* by directed molecular evolution

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Several techniques in directed molecular evolution have emerged as powerful tools to increase the activity and stability of several proteins. These techniques are based on introducing random mutations either by the recombination between DNA homologous sequences (DNA shuffling) or, by introducing random copying errors by imposed imperfect DNA polymerase activity (Error-prone PCR). The objective of these techniques is to determine by a screening method which of the thousands of mutated genes can be expressed, tested, and selected by choosing the best product with a specific characteristic. In this work we used DNA shuffling for three homologous genes that encoded Cry11 toxins produced by three subspecies of *Bacillus thuringiensis* (Bt). The genes *cry11Aa* (Bt. *israelensis*), *cry11Ba* (Bt. *jegathesan*) and *cry11Bb* (Bt. *medellin*) were isolated by PCR in order to proceed with random DNA fragmentation by DNaseI. After reassembling and assembling, the products obtained were around 0.75, 2.5 and 3 kb. These fragments were cloned and 93 positive clones were obtained from which 10% showed high homology with *cry11Aa*. Further analysis are carrying out to determine their expression in a non crystal producer strain of

B. thuringiensis in order to evaluate their toxicity against *Anopheles albimanus, Aedes aegypti* and *Culex quinquefasciatus*.

Contributed paper. Thursday, 14:15. **201**

Characteristics of a *sigL* mutant in *Bacillus thuringiensis* HD-73

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A *sigL* gene deactivation insertion mutant was obtained and its genetically complementary strain was also constructed. The *sigL* mutant could not grow in the basic medium with arginine, proline, valine, isoleucine, glutamine, phenylalanine, methionine and tryptophane as the sole nitrogen source separately. The *sigL* mutant strain retarded the formation of crystal protein Cry1Ac, comparing to the host strain HD-73. The amount of live spore in the *sigL* mutant strains was less than that in HD-73 host strain. We constructed the promoter of *gabT* gene and *lacZ* fusion expression vector. Assay of β-galactosidase activity showed that transcription of *gabT* gene was decreased in *reg* gene (downstream of *gabT* gene) mutant and *sigL* mutant. This result indicated that *sigL* regulated the GABA pathway and the upstream sequences of *gabT* were regulated by the *reg* gene. We also constructed the promoters of *acoR and bkdR* gene and their corresponding *lacZ* fusion expression vectors. β-galactosidase assay revealed that AcoR and BkdR are SigL-dependent transcriptional activators in *Bacillus thuringiensis* strains, probably the operons which were regulated by AcoR and BkdR were also controlled by SigL respectively.

Contributed paper. Thursday, 14:30. **202**

The characteristics of an antagonistic *Bacillus thuringiensis* strain against crop pathogens and pests

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Bacillus thuringiensis strain 519-1 were tested for the antagonistic activity against the growth of hyphae of eight fungi including *Aspergillium niger, Botrytis cinerea, Fusarium graminearum, Penicillium chrysogenum, Physalospora piricola, Rhizoctonia solani, Rhizopus nigricans*. It could notably inhibit sporangia germination of all the tested fungi. The culture of 519-1 exhibited high toxicity against *Helicoverpa armigera* and *Spodoptera exigua*, with LC_{50} values of 12.8μg/ml and 5.5μg/ml, respectively. PCR analysis with specific primers showed that the strain contained five insecticidal protein encoding genes: *cry1Aa, cry1Ab ,cry1Ac, cry2, cry1I*, and a vegetative insecticidal protein gene, *vip3A*. Cry proteins with molecular weight about 130,80,75,65 and 60kDa were detected using SDS-PAGE. The results showed that Bt519-1 was a strain which had high activities of broad-spectrum antagonistic and high insecticidal toxicity against lepidopteran pests.

Contributed paper. Thursday, 14:45. **203**

Characterization of mosquitocidal *Bacillus cereus* toxic to *Ochlerotatus taeniorhynchus* and *Culex quinquefasciatus*

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Two spore-forming bacteria, M413 and C32, were isolated from sediment samples collected in Florida. The bioassay results showed that M413 is mainly toxic to *Ochlerotatus taeniorhynchus* whereas C32 is to *Culex quinquefasciatus*. M413 was not active against *Aedes aegypti* and *Cx. quinquefasciatus*, and showed low toxicity against *Anopheles quadrimaculatus*. Interestingly, it was not active against *Ae. aegypti*, a closely related species of *Oc. taeniorhynchus*. C32 did not show any toxicity against *Ae. aegypti* or *An. quadrimaculatus*, but showed low toxicity against *Oc. taeniorhynchus*. Gas chromatographic analysis of fatty acids methyl esters and 16S rRNA gene sequence alignment revealed that M413 has 0.74% genetic difference and the Similarity Index of 0.846 with *Bacillus cereus*. C32 also has 0.74% genetic difference and the Similarity Index of 0.845 with *B. cereus*. Although M413 and C32 do not produce any crystals, both produced two distinct major proteins of ca. 97 and 30 kDa. All four proteins showed the highest homology with the 93-kDa S-layer protein of *Bacillus licheniformis* using liquid chromatography – mass spectroscopy / mass spectroscopy, suggesting that the 30-kDa proteins from both isolates may be degradation products of the 97-kDa proteins. Novel proteins identified could be useful to engineer new types of recombinant mosquitocidal bacteria.

Contributed paper. Thursday, 15:00. **204**

Pathogenesis of male-killing *Wolbachia* in *Drosophila bifasciata*

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Wolbachia are a common and widespread group of bacteria found in a wide range of arthropods. These bacteria are transmitted through the cytoplasm of eggs and have evolved various mechanisms for manipulating reproduction of their hosts, including induction of reproductive incompatibility, pathenogenesis, male-killing and feminization. In *Drosophila bifasciata*, it causes male death during embryogenesis. We have investigated this male-killing phenotype using TUNEL assay and anti-SXL antibody (*Sex-lethal*, the master regulator of sex determination, expressed only in females) on infected and uninfected embryos. Male embryos do not express *Sex-lethal* at any point and appear to develop normally up to stage 11 of embryogenesis. However, a strong and widespread apoptosis is observed in the subsequent stages in infected males while a normal localized pattern is observed in infected females as well as in uninfected males and females. Anti-WSP (*Wolbachia* surface protein) antibodies revealed that this sex specific virulence is not associated with excessive bacterial replication. We will discuss these results with respect to other *Wolbachia*-host interactions, in addition to other bacterial infections that cause the same phenotype

THURSDAY PM

Contributed paper. Thursday, 15:15. **205**

Brevibacillus laterosporus* potential against the house fly and its safety for the non-target pupal parasitoid *Muscidifurax raptor

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The potential of various *Brevibacillus laterosporus* strains as biological control agents against different insect pests has recently been demonstrated. Our studies have highlighted a new and very promising strain showing toxicity against the house fly *Musca domestica*. Our laboratory observations suggest that the pathogenic activity of this bacterial strain for *M. domestica* is a toxin-mediated process reminiscent of the mechanism of action of *B. thuringiensis* δ-endotoxins. Major proteins, with a molecular weight of about 14 kDa, extracted from the *B. laterosporus* typical canoe-shaped parasporal body, are involved in the observed toxicity. On the other hand, the employment of any biological control method is strictly dependent on its safety for naturally occurring biological control agents. Interestingly, when our *B. laterosporus* strain was assayed at high concentration on adults of one of the main house fly parasitoids, *Muscidifurax raptor*, only slight effects were noticed. In addition, no tritrophic interaction (house fly-bacteria-parasitoid) was detected. Therefore from every aspect, the compatibility of this *B. laterosporus* strain in house fly integrated management strategies with parasitoids is promising.

CONTRIBUTED PAPERS Thursday, 14:00-15:45

MICROBIAL CONTROL 3

Contributed paper. Thursday, 14:00. **206**

Toward aphid-resistant transgenic plants

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While transgenic plants expressing *Bacillus thuringiensis* (Bt)-derived toxins have met with widespread success for management of lepidopteran and coleopteran pests, Bt-derived toxins are not effective for management of the sap-sucking insects within the order Hemiptera. Indeed, in some instances damage caused by hemipteran pest species which include aphids and plant bugs, has compromised the success of the Bt-based technology. Plant viruses which are transmitted by aphids in a persistent, circulative manner enter the aphid hemocoel by a receptor-mediated process. We have shown that the coat protein (CP) of such a virus, *Pea enation mosaic virus* (PEMV: *Luteoviridae*), when fused to an effector protein delivers the effector protein into the aphid hemocoel. For example, a CP-P-EGFP fusion protein with a proline-rich linker derived from the virus (-P-) was delivered into the aphid hemocoel. Uptake of this fusion protein showed that the virion structure is not required for uptake of CP from the aphid gut. PEMV CP fused to the spider-derived insecticidal toxin w-atracotoxin-Hv1a was tested for aphicidal activity by using membrane feeding assays with *E. coli*-expressed fusion proteins, and by transient expression of fusion proteins in *Nicotiana benthamiana*. These experiments show promise for the use of this approach for production of aphid-resistant transgenic plants.

Contributed paper. Thursday, 14:15. **207**

***Yersinia* n. sp. EN65 a novel insecticidal bacterium: A new biocontrol agent for diamondback moth, *Plutella xylostella*?**

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Diamondback moth (DBM) is a significant pest of crucifer crops worldwide. It is resistant to many older pesticides (e.g., organophosphates, pyrethroids), newer chemistries (e.g., Spinosad), and biopesticides based on *Bacillus thuringiensis* (*Bt*) toxins, creating a need for new compounds and microbial control agents. Laboratory tests have demonstrated that the non-spore forming bacterium *Yersinia* n. sp. (tentatively *entomophaga*) EN-65 strain is active against a range of coleopteran and lepidopteran pests, including DBM. Here, we report on a study to characterise the insecticidal activity of EN-65 against a susceptible strain of DBM. For comparison, two 'standard' products were included in the trial: Dipel™ (based on *Bt* var. *kurstaki* - *Btk*) and Success Naturalyte™ (Spinosad). Excellent control of DBM was achieved on infested cabbage plants sprayed with the bacterium. Although Spinosad gave superior control, EN-65 performance was comparable to Dipel. When applied as a sprayable gel formulation, higher leaf deposition rates and persistence were obtained compared to a basic broth culture spray. A gel formulation could also serve as a vehicle for inclusion of other formulation additives that may prolong residual activity in open field conditions. Finally, EN-65 multiplied within DBM cadavers, potentially serving as a source of infection for feeding larvae beyond that of the original spray. In addition to providing basic efficacy and persistence data, the findings have implications regarding potential survival and recycling of EN-65 within a pest population.

Contributed paper. Thursday, 14:30. **208**

Biochemical characterization and insecticidal activity of an alkaline metalloprotease produced by *Photorhabdus luminescens* 0805-P5G isolated from Taiwan

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Proteases play a key role in the interaction between pathogens and their hosts. Among 13 local *Photorhabdus luminescens*, 4 candidates with higher proteolytic activity assayed with skim milk and gelatin plates were selected for further study. Three liquid cultures namely, NB, LB and PP3T were chosen to cultivate the candidates and their proteolytic activities were measured. Results showed that after 8-day liquid cultured in NB medium, the proteolytic activity of isolated *P. luminescens* 0805-P5G reached to the maximum. The activity of protease from *P. luminescens* 0805-P5G was greatly increased after a serial purification process by FPLC using DEAE-Sepharose column and Q sepharose column. Zymograpphic analysis was applied to confirm the molecule weight of protease, approximately 54 kDa, which was further reconfirmed by SDS-PAGE. Moreover, the peptide sequence of protease of *P. luminescens* 0805-P5G was identified by N-terminal sequencing analysis and MS/MS. The activity of purified protease was optimum at 60°C and pH 8. Bioassay of *P. luminescens* protease against *Galleria mellonella* by injection showed that it has high insecticidal activity. High oral toxicity for *Plutella xyllostella* (Taiwan strain) was also found, however, the oral toxicity for *Plutella xyllostella* American strain was low. The protease was inhibited by EDTA and 1, 10-phenanthtoline and categorized as metalloprotease.

Contributed paper. Thursday, 14:45. **209**

Heterologous expression of recombinant bacterial endochitinases and production of chitin-derived oligosaccharides

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The objective of the study was to synthesize two heterologous endochitinases in *Escherichia coli* and demonstrate their potential for applied use in generating antimicrobial oligosaccharides (OGS) derived from chitin. Native endochitinase genes, *chiA NIMA* from *Serratia marcescens* and *chiA74* from *Bacillus thuringiensis*, were cloned into two vectors for heterologous expression in *E. coli*. Without modifications of these genes, the corresponding encoded endochitinases were secreted by the *E. coli* protein export machinery, and by ~ 20 hours, maximal chitinolytic activities were observed. The highest activity using colloidal chitin as the substrate was observed with ChiA NIMA, which produced OGS with different degrees of polymerization. Antimicrobial activities against *Enterobacter cloacae*, *Staphylococcus aureus*, and *S. xylosus* were observed with crude OGS preparations obtained after ChiA NIMA digestion of chitin. Our study suggests that it is feasible to synthesize ChiA NIMA and ChiA74 in *E. coli* and mass produce these enzymes in culture supernatants for applied use. In addition, as signal peptides in native ChiA Nima and ChiA74 were recognized by the molecular export apparatus in *E. coli*, these short peptides could be included in other commercially produced recombinant proteins that are heretologously synthesized in *E. coli*.

Contributed paper. Thursday, 15:00. **210**

Plusiine baculoviruses: Potential for cabbage looper, *Trichoplusia ni*, control in greenhouse vegetable production

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The cabbage looper, *Trichoplusia ni*, has become a serious pest of greenhouse vegetable production in Canada due in part to the development of resistance to *Bacillus thuringiensis* (Bt) based bioinsecticides. Most Canadian greenhouse vegetable production is chemical pesticide free, relying on biological control agents deployed in an IPM system for insect pest control, thus alternatives to Bt products are required. Indigenous strains of TnSNPV and AcMNPV were selected based on laboratory assessments of infectivity and virulence. Selected strains were evaluated as microbial pesticides in a series of cage and open compartment greenhouse spray trials on cucumber plants artificially infested with *T. ni* larvae. Preliminary dose uptake spray trials with 2nd and 4th instar larvae showed high levels of infection and mortality with both TnSNPV and AcMNPV at dose levels ranging from $5.0x10^{10}$ to $1.0x10^{12}$ OB/Ha equivalents (400L/Ha). Subsequent caged trials demonstrated greater than 90% mortality in 2nd instar populations by nine days post treatment for both TnSNPV and AcMNPV isolates. Finally, a larger scale spray trial was conducted on cucumber plants, seeded with 2nd instar *T. ni*, sprayed with the AcMNPV isolate at $1.0x10^{12}$ OB/Ha (400L/Ha volume equivalent) and destructively sampled at 2, 6, 12 and 14 days post treatment. The AcMNPV treated plots showed significantly lower numbers of surviving larvae and lower foliage and fruit damage at 6 days post treatment and onwards. These initial results indicate good potential for indigenous baculovirus isolates as biological control agents of *T. ni* in greenhouse vegetable production.

Contributed paper. Thursday, 15:15. **211**

Use of a granulovirus (PoGV) and *Bacillus thuringiensis* (Bt) to control potato tuber moth (*Phthorimaea operculell*)

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Progress in this project includes in vivo production for PoGV, and successful field testing of PoGV and Bt under field and storage conditions. Field studies showed that although PoGV does not immediately kill potato tuber worm (PTM) larvae, it controls future generations by preventing breeding, because infected larvae completing larval development fail to pupate. For example, in 2006 weekly virus treatments in field cages caused a 76.3% reduction in mined leaves, and a 96.3% and 97.4% reduction of PTM larvae recovered from foliage and artificially added tubers, respectively, in the 2nd generation compared with controls. In storage studies, we have tested PoGV and Bt incorporated with various dry carriers (sand, talc, diatomaceous earth and kaolin) as a method to control PTM in stored tubers. Tubers can alternatively be dipped in test suspensions and dried prior to storage. Our data show applications were very effective against neonates at very low rates (e.g. 1 larval equivalent of PoGV can treat over 100 kg tubers), but less effective against larvae already inside tubers, which requires higher rates to kill larvae. In general a successful strategy for these microbials would be to prevent the spread of any suspected infestation in storage. One advantage is that these agents persist for long periods of time under cool and dark conditions of storage. This latter strategy is currently being tested in scaled up studies, including an evaluation of the effect of incubation temperature.

Contributed paper. Cancelled **212**

Contributed paper. Thursday, 15:30. **213**

Finding a microbial control agent for the invasive crayfish, *Orconectes virilis*

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Arizona has no native crayfish. *Orconectes virilis* was introduced into Arizona almost 40 years ago for bait and food. These crayfish have had a devastating effect on stream and pond habitats wherever they have been introduced, removing native vegetation, and are associated with declines in populations of native fish, snails, insects, snakes, turtles and amphibians. Removal of these crayfish is a major target of Arizona Game and Fish Department. We developed an alginate pellet technique for delivery of the potential pathogens to the target crayfish. We tested 21 species or strains of insect pathogenic Bacillus including 6 strains of *Bacillus thuringiensis*, 7 bacterial strains isolated from sick crayfish, 4 species of insect parasitic nematodes, and White Spot Syndrome Virus (WSSV) from marine shrimp as possible biological control agents for *O virilis*.

Only the shrimp pathogen, WSSV, proved to be pathogenic for *O virilis* . WSSV could be passed by cannibalism but not by water. WSSV was further tested against 2 species of freshwater crustaceans found in the local habitats, and was not found to be pathogenic to these nontarget organisms.

SYMPOSIUM (Microbial Control) Thursday, 16:30-18:30

Regulatory and Market Barriers for Approval of Microbial Control Products

Cancelled: Symposium. Thursday, **214**

Symposium. Thursday, 16:30. **215**

Regulatory innovation and biopesticide commercialisation

Wyn P. Grant[1]; Justin G. Greaves[1]; David Chandler[2]; Gillian Davidson[3]; G Mark Tatchell[3]
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Microbial biopesticides can make important contributions to IPM, but their commercialisation is dependent upon the regulatory system that governs their authorisation. Regulations based on those for chemical pesticides can act as a barrier to commercialisation. Although there is a strong role for government in helping new industries that bring positive public benefits, regulatory authorities have a difficult job to ensure product quality and safety while not inhibiting commercialisation. We have been investigating the regulation of microbial biopesticides in the UK, although our work is relevant generally. The UK regulator, the Pesticides Safety Directorate, introduced a biopesticides Pilot Project in 2003 and converted this into a Biopesticides Scheme in 2006. Our study of this process helped us develop a model specifying the conditions under which regulatory innovation can occur. We have also investigated the role of retailers in biopesticide governance. Major supermarket chains consider that they are under pressure from consumers to minimise pesticide residues. This leads them to prohibit pesticides that have been approved by the regulatory system. However, they are reluctant to recommend the wider use of biopesticides as alternatives. Thus private governance is likely to provide a barrier to market entry for biopesticide products.

Symposium. Thursday, 17:00. **216**

Microbial control products: The regulatory challenge

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Traditionally in the UK only a small number of approved biopesticide-type products have been available in market place; pre-2003 there were four actives. Therefore, following discussions with growers, manufacturers and policy-makers the UK pesticide regulatory body (PSD) launched a Biopesticides Pilot Project in June 2003. This was seen as a way of learning more about this sector, encouraging potential applicants to open dialogues with PSD and also offered reduced fees; often quoted as the main obstacle to product availability. It resulted in three new products being successfully approved containing pheromone, viral and fungal active substances. Building on the success of the Pilot Project; 2006 saw PSD launch a full-scale UK Biopesticides Scheme. The aim of this is to increase the number of products entering the market that are alternatives to conventional pesticides. The scheme covers a range of biopesticides including products containing micro-organisms and has now resulted in ten biopesticides being approved in the UK. The presentation provides background on the Biopesticide Scheme and possible future developments in the EU. It also touches on the challenges for regulators aiming to provide as much flexibility as possible whilst looking to provide regulation at an appropriate level.

Symposium. Thursday, 17:30. **217**

Commercialization of microbial control products: The industry perspective

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Advancement made since 1995 in science and in the development of realistic marketing and sales strategies increased growers acceptance for microbial control products. The lesson learned from earlier problems have encouraged leading companies to improve cost efficiency of their technologies and adopt better product positioning within the confines of product capabilities. To sustain profitable business, in depth planning to enhance product sales is needed through IPM programs, new formulations, new delivery systems, reliable quality control and training programs. The market for microbial insecticides is approximately US$268 million, which represents approximately 1.5% of the total crop protection insecticide market, and most of this is due to sales of Bt products (US$160 million). However, the market potential for the microbial insecticides will likely increase in the next few years due to the following: An increase in the awareness of the growers, distributors and farm advisors about the benefits and the flexibility of including microbial insecticides in IPM programs. The producers of microbial insecticides are positioning their products as partners and not as alternatives to chemical insecticides. The demand of the regulatory agencies and the general public for safer produce and for products with no detrimental effect to the environment. High probabilities of insect resistance developing against reduced-risk insecticides.

Symposium. Thursday, 18:00. **218**

Understanding the adoption of alternative pest management strategies: An economist's view

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Agriculture faces a serious challenge to develop systems of plant protection that are economically, environmentally and socially sustainable. At present, most plant protection against agricultural pests relies heavily on chemical pesticides. These are among the most highly regulated of chemicals, but there are still legitimate concerns about their external costs. Left unchecked, externalities drive a wedge between freely determined market prices and socially optimal prices. There is a clear rationale, therefore, for interventions that reduce externalities and to ensure market prices internalise those externalities that remain. In this presentation, the impediments to the adoption of biologically-based alternatives to chemical pesticides will be considered from a socio-economic point of view. Lack of knowledge in these areas is acting as a barrier to the development of sustainable agriculture in Europe and elsewhere. Over reliance on chemical pesticides could be remedied in part by substitution with alternative plant protection technologies, including microbial control products, done as part of Integrated Pest Management. However, if substitution is to be a legitimate way forward, then a new understanding is required of the external costs of pesticides, the costs and benefits of alternatives, and the effectiveness of policy instruments used to facilitate substitution.

CONTRIBUTED PAPERS Thursday, 16:30-18:30

BACTERIA 5

Contributed paper. Thursday, 16:30. **219**

B.t.-toxins in the midgut of Western corn rootworm (*Diabrotica virgifera virgifera* LeConte)

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The Western corn rootworm (WCR) is one of the economical most important pests in corn. For its control, genes encoding *Bacillus thuringiensis* toxins (e.g. Cry3Bb1, Cry3A, Cry34Ab1/Cry35Ab1) were introduced into corn. The cultivation of transgenic corn expressing the respective *B.t.*-toxins may result in the development of resistant pest populations. In general, the resistance of insects to *B.t.*-toxins can be located at any step of the toxic pathway. However, in other *B.t.*-toxin-pest-systems, the resistance mechanisms are mainly proteinase- or receptor-mediated. To establish reference systems for the identification of resistance mechanisms in potential available resistant individuals, studies on proteinase activities and binding analysis were carried out with midgut fluid and midgut epithelium of WCR 3rd instar larvae. Studies on the identification and quantification of proteinase activities in the midgut fluid were conducted using photometrical tests with specific chromogenic substrates - mainly peptidyl-*p*-nitroanilid (*p*NA) - and specific inhibitors. As a result, the digestive serine endopeptidases trypsin, chymotrypsin, and elastase were identified. Besides, high digestive activities were observed for the serine endopeptidases cathepsin G, plasmin, and thrombin. Due to the acid midgut fluid, in *Chrysomelidae* cysteine endopeptidases were expected. Accordingly, high activities of cathepsin L, papain, cathepsin B, and cathepsin H were observed in the midgut fluid of WCR (pH 5.75). Besides, the metallo endopeptidase saccharolysin as well as the exopeptidases aminopeptidase and an omegapeptidase - acylaminoacylpeptidase - were identified. For aspartic endopeptidases no specific *p*NA substrates were available. Using the general proteinase substrate azocasein, the activity of the aspartic endopeptidase pepsin was demonstrated. Furthermore, with midgut epithelium binding analysis were carried out to study binding site competition of *B.t.*-toxins Cry3Bb1 *vs.* Cry34Ab1/Cry35Ab1. From the midgut epithelium brush border membrane vesicles (BBMV´s) were prepared. To examine the toxin binding, biotin labeled *B.t.*-corn-toxins, and the ligand-blot technique as well as streptavidin-horseradish-peroxidase-conjugat and the ECL system were used.

Contributed paper. Thursday, 16:45. **220**

Mutations in the *cadherin* gene in a *O. nubilalis* strain selected for Cry1Ab resistance.

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An *Ostrinia nubilalis* colony was selected for resistance to *Bacillus thuringiensis* Cry1Ab protoxin. Previous work evidenced the implication of more than one genetic locus and the reduction of the cadherin receptor. We have now determined the contribution of the *cadherin* gene to the overall Cry1Ab resistance in this strain. Individual larval midguts from susceptible (Europe-S) and resistant (Europe-R) insects were used to prepare cDNAs from the *cadherin* gene. We found major mutations that suggested highly structural deficient proteins because they introduced premature termination codons (PTC) and/or large deletions (1383-1701 bp). In the resistant strain, these mutations were found in 13 out of 20 insects analyzed. In the susceptible strain, only one PTC was detected among the major mutations, but always in heterozygotes. To check for the contribution of the major mutations to the resistance, Europe-R insects were subjected to a high dose of Cry1Ab protoxin. The analysis of the survivors showed that major mutations were absent. These results support a polygenic inheritance of resistance in the Europe-R strain, in which mutations in the *cadherin* gene would contribute to resistance by means of an additive effect.

Contributed paper. Thursday, 17:00. **221**

Bacillus thuringiensis Cry2A toxins bind saturably to a common site in the midgut of *Helicoverpa armigera*

C. Sara Hernández-Rodríguez[1]; Adri Van Vliet[2]; Nadine Bautsoens[2]; Jeroen Van Rie[2]; Juan Ferré[1]
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For a long time, it has been assumed that the mode of action of Cry2A toxins was unique due to the apparent non-specific and non-saturable binding to a practically unlimited number of membrane receptors. However, this assumption seems to be in contrast with the highly homologous tertiary structure among the 3-domain Cry toxins, including Cry2A toxins. To verify the existing data on the particular mode of action of Cry2A toxins, binding assays were carried out with ^{125}I-Cry2Ab and ^{125}I-Cry1Ac. Saturation and competition assays showed that Cry2Ab does bind with high affinity, in a specific and saturable manner, to brush border membrane vesicles of *Helicoverpa armigera* and *H. zea*. Heterologous competition assays in *H. armigera* showed the occurrence of a common binding site for three toxins belonging to the Cry2A family (Cry2Aa, Cry2Ab, and Cry2Ae), but not for Cry1Ac. Our results question interpretations of published data of binding assays with Cry2A toxins from other authors and establish the basis of the mode of action of Cry2A toxins.

Contributed paper. Thursday, 17:15. **222**

The importance of antibiosis and inter-specific competition in the ecology of *Bacillus thuringiensis*

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Understanding that factors limiting pathogen growth and fitness can give important insight into improving their use in pest control. Here, we investigated to what extent inter-specific competition with other micro-organisms determines the biology and ecology of Bacillus thuringiensis. Firstly, we examined the distribution and expression of antibiotic genes (zwittermicin a) in pathogenic and non-pathogenic members of the Bacillus cereus group using PCR and phenotypic assays of virulence and antibiosis. Secondly, we passaged a B. thuringiensis strain derived from DiPel (Btk rifR) with low antibiotic expression through larvae of the diamondback moth, Plutella xylostella, and tested for changes in levels of antibiosis. We found that levels of expressed antibiosis and positive amplification of an antibiotic gene (zwittermicin orf7) were very good predictors of whether strains expressed bi-pyrimidal toxin crystals. These traits were better predictors of toxin expression than possession of cry genes since many strains that possessed cry genes failed to express toxins. Passage of Btk rifR through P. xylostella resulted in significant increases in levels of detectable antibiosis in three independent lineages. We conclude that antibiosis and inter-specific competition are important factors for the successful exploitation of hosts by pathogenic members of the B. cereus group.

Contributed paper. Thursday, 17:30. **223**

REPAT proteins and their role in the tolerance of *Spodoptera exigua* to its pathogens

Carmen S. Hernandez[1]; Patricia Hernandez-Martinez[1]; Gloria Navarro-Cerrillo[1]; Juan Ferre[1]; Baltasar Escriche[1]; William J. Moar[2]; Ruud A. de Maagd[3]; Salvador Herrero[1]
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The response of insects to pathogens involves changes in gene expression, which may help the insect to overcome the infection by the pathogens or the effect of their toxic compounds. Studying the response of *Spodoptera exigua* to its pathogens we detected a novel family of genes that were up-regulated after larval exposure to different *B. thuringiensis* toxins and also during the infection with the baculovirus *Autographa californica* (Ac)MNPV. These genes, due to their expression in **res**ponse to **pat**hogen, were called *Repat* genes. So far, we have detected 8 members of this family, all coding for proteins with a predicted molecular weight of approximately 15-20 KDa. Characterization of the genomic structure of 2 of the most distant members of the *Repat* family has revealed a similar organization, suggesting a common origin for the different members. In the present work we summarize our recent advances in the determination of the molecular function of REPAT proteins. We also report here current evidences supporting the role of REPAT proteins in attenuating the pathological effects of *B. thuringiensis* and baculovirus

Contributed paper. Thursday, 17:45. **224**

Cloning and expression of the Cry1Ac-binding alkaline phosphatase (HvALP) from *Heliothis virescens*

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We previously reported the identification of an alkaline phosphatase (HvALP) that bound Cry1Ac toxin in brush border membrane vesicles (BBMV) from *Heliothis virescens*. Lower alkaline phosphatase-specific activity and HvALP protein levels in Cry1Ac-resistant larvae suggested a functional role for this protein in the Cry1Ac intoxication process. In this work we report the cloning of three isoforms of membrane bound alkaline phosphatase (mALP) from *H. virescens* larval midgut. These three isoforms share high sequence identity, although they also present distinctive features. Analysis of expression confirmed that although alkaline phosphatases are present in a number of larval tissues, all three mALP clones are only expressed in the foregut and midgut epithelium. Heterologously expressed mALP isoforms were used to study interactions with Cry1Ac toxin and characterize the role of HvALP in Cry1Ac intoxication.

Contributed paper. Thursday, 18:00. **225**

Cloning of a Cry3Aa-receptor cadherin from *Tenebrio molitor*

Jeff Fabrick[1]; Cris Oppert[2]; Marcé Lorenzen[3]; Brenda Oppert[3]; Juan Luis Jurat-Fuentes[2]
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Cry toxins produced by the bacterium *Bacillus thuringiensis* (Bt) are effective biological insecticides. Cadherin-like proteins serve as functional receptors for these toxins in Lepidoptera, but little is known in Coleoptera. We present the first report demonstrating a functional interaction between the coleopteran-specific Cry3Aa toxin and a coleopteran cadherin. This putative Cry3Aa receptor cadherin was cloned from *Tenebrio molitor* larval midgut mRNA and the predicted protein, TmCad1, shares similarity with lepidopteran cadherin Bt receptors in both domain structure and putative toxin binding region. A TmCad1 peptide (rTmCad1p) containing the putative toxin binding region specifically interacted with Cry3Aa and promoted the formation of Cry3Aa toxin oligomers. Moreover, functional analysis demonstrated that TmCad is a toxin binding protein that promotes Cry3Aa toxicity in *T. molitor* larvae. Our data suggests similarities between the mode of action of Cry toxins in Lepidoptera and Coleoptera.

Contributed paper. Thursday, 18:15. **226**

***Bacillus thuringiensis* camelysin accumulates in biofilm and is also in vivo expressed**

Thomas Candela[1]; Christophe Buisson[2]; Nathalie Gilois[2]; Stéphane Aymerich[3]; Didier Lereclus[2]; Christina Nielsen-LeRoux[2]; Michel Gohar[1]
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A high proportion of B. thuringiensis or B. cereus strains produces biofilms in various culture media and on diverse surfaces, mostly at the liquid-solid interface. When established as biofilms, bacterial populations are known to be more resistant to antimicrobial agents and to environmental stresses. As such, biofilms may be involved in the bacterial pathogenesis when they occur within the host as they may protect the bacteria from host defence factors. Most of the virulence factors produced by B. cereus are controlled by the transcriptional regulator PlcR. Using transcriptional fusions, we have followed PlcR expression during biofilm formation and we found that it peaks at after 72hrs. To check the presence of virulence factors in B. cereus biofilms we investigated the production by proteomic analysis using 2-dimensional gel electrophoresis and MALDI-TOF. Camelysin (CalY), a surface protein, was highly accumulated in the biofilm. In vivo studies, based on gfp-promoter fusion in Galleria mellonella (Gm) larvae, confirmed expression during infection as well. A caly mutant strain [Bt-407Cry-Δcaly] turned out to be slightly reduced in virulence towards Gm and was also affected in adhesion to a eukaryotic cell cultures. We are actually investigating on biofilm formation in vivo.

CONTRIBUTED PAPERS Thursday, 16:30-18:15

VIRUSES 6

Contributed paper. Thursday, 16:30. **227**

"Here's spitting at you, kid" - Oral transmission of the *Musca domestica* salivary gland hypertrophy virus (MdSGHV) via salivary secretions

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The *Musca domestica* salivary gland hypertrophy virus (MdSGHV) is a newly characterized, double stranded DNA virus that replicates in the salivary glands of infected adult house flies. This non-occluded, enveloped virus is believed to be orally transmitted within feral populations of *M. domestica*. Droplet-feeding of individual flies with viremic salivary gland homogenate demonstrated an age-dependent susceptibility of the adults to viral infection. Challenging flies at 1, 6, and 24 h post-emergence resulted in an average 50%, 5%, and 0% infection, respectively. Using quantitative real-time PCR, MdSGHV was quantified in saliva samples obtained from individual viremic flies. Beginning with the onset of SGH symptoms at 3 d post-injection, an average 10^5 to 10^6 viral copies were released per fly per feeding event. Saliva transmission experiments showed that the released virus was infectious when ingested by 1-h old adult flies, resulting in an average 66% infection rate. Again, susceptibility to viral infection was almost completely reduced when flies were challenged with salivary secretions at 6 h and 24 h post-emergence. Potential factors that could be responsible for the age-related resistance to per os infection will be discussed.

Contributed paper. Thursday, 16:45. **228**

MdSGHV transcriptome during viral infection in the house fly

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The *Musca domestica* salivary gland hypertrophy virus (*Md*SGHV) is a nonoccluded, enveloped, rod-shaped and double stranded DNA virus that has been characterized by its ability to induce enlarged (hypertrophied) salivary glands in adult house flies. MdSGHV was detected and isolated from hypertrophied salivary glands of male and female houseflies, *Musca domestica* L., in Florida. The genome of MdSGHV has recently been sequenced (GenBank Acc. No.EU522111). The putative open reading frames (ORFs) showed similarity to *Glossina pallidipes* SGHV, however, these ORFs have not been validated as true transcripts. In an effort to know more about the transcriptome of this newly sequenced virus in house fly cells, Rapid Amplification cDNA Ends (RACE) was performed mainly on the 3' terminus of MdSGHV transcripts (3'RACE) and/or the 5' terminus (5'RACE). The information of the three prime untranslated regions (3'UTRs) has led to rearrangement of some of the putative ORFs on the MdSGHV genome. Validating these putative ORFs was important since most of them did not show homology to any gene references in the GenBank. The up- and down-regulation of these validated ORFs will be addressed in this study.

Contributed paper. Thursday, 17:00. **229**

Isolation and functional analysis of an ascovirus-encoded microRNA regulating viral replication

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MicroRNAs (miRNAs) are small (~22 nucleotide) non-coding RNAs which play an essential role in gene regulation, and affect a wide range of processes including development, differentiation, and oncogenesis. Here we report the isolation of the first miRNA from an insect virus, which is encoded within the major capsid protein (MCP) gene in *Heliothis virescens* ascovirus (HvAV) (hvav-miR-1). Although MCP is highly expressed at all time points 24 hours after infection, hvav-miR-1 expression is tightly regulated and specifically detected from 96 hours post infection. Hvav-miR-1 expression coincides with a marked reduction of HvAV DNA polymerase I, which is a predicted target. Indeed, ectopic expression of full-length and truncated versions of MCP retaining the miRNA sequence reduce DNA polymerase levels and inhibit viral replication. Our results indicate that hvav-miR-1 may be a key regulator of HvAV replication.

Contributed paper. Thursday, 17:15. **230**

Immobilization of proteins into *Bombyx mori* cypovirus polyhedra

Hajime Mori[1]; Hiroshi Ijiri[1]; Gento Nishimura[1]; Takeshi Nakatani[1]; Keiko Ikeda[2]; Fasseli Coulibaly[3]; Elaine Chiu[3]; Peter Metcalf[3]
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Cypoviruses and baculoviruses are notoriously difficult to eradicate because the virus particles are embedded in micrometresized protein crystals called polyhedra. The remarkable stability of polyhedra means that, like bacterial spores, these insect viruses remain infectious for years in soil. We have determined the 2Å crystal structure of the polyhedrin of *Bombyx mori* cypovirus (BmCPV). We found that polyhedra are made of trimers of the viral polyhedrin protein and contain nucleotides. Although the shape of these building blocks is reminiscent of some capsid trimers, polyhedrin has a new fold and has evolved to assemble *in vivo* into threedimensional cubic crystals rather than icosahedral shells. The fold of polyhedrin has the shape of a left hand with the thumb and index finger outstretched. The index finger is an N-terminal a-helix (H1) which extends from the fist formed by a compact three-layer b sandwich core. We developed a new method for immobilization of foreign protein into the CPV polyhedra by use of the H1 sequence. Cell growth factor (FGF-2 and FGF-7) and enzyme (protein kinase C) were immobilized into the polyhedra and their biological activities were compared with those which were immobilized by our conventional method using the N-terminal sequence of BmCPV VP3.

Contributed paper. Thursday, 17:30. **231**

Flies infected with *Wolbachia* are less susceptible to *Drosophila C virus*

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Insect responses that are specific for virus infection have been investigated in *Drosophila melanogaster*. Most studies focus on interactions with *Drosophila C virus* (DCV), a member of the *Dicistroviridae* family. Several genes controlled by the Jak-STAT pathway are upregulated upon DCV infection. To investigate the host:virus interactions that induce these responses we used the Jak-STAT regulated gene *vir-1* as a reporter gene. We challenged several different strains of *Drosophila* with DCV and identified one strain in which the *vir-1* gene was not upregulated. Interestingly, flies of this strain were also more resistant to DCV induced mortality. Treatment of the flies with tetracycline abrogated the resistance phenotype, suggesting that resistance may be conferred by bacterial infection. PCR screening indicated that the resistant flies were infected with the common intracellular symbiont *Wolbachia*, whereas tetracycline treated flies were *Wolbachia* free. Challenge of further fly strains infected with *Wolbachia* and comparison with paired tetracycline treated flies indicated that the resistance phenotype was linked to *Wolbachia* infection status. Given more than 20% of all insect species are thought to be infected with *Wolbachia*, it will be important to determine if these results are DCV specific or the interaction extends to other virus groups.

Contributed paper. Thursday, 17:45. **232**

Pathological effects and possible ecological impact of newly identified viruses of the aphids *Brevicoryne brassicae* and *Dysaphis plantaginea*

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The impact of viruses on the aphids physiology and population dynamics remains poorly understood despite their potential ecological and economic importance. Using a method for sequence-unbiased amplification of viral nucleic acids, we have demonstrated the existence of viral diversity in economically important aphids species. We have identified and sequenced a virus from the cabbage aphid (*Brevicoryne brassicae* virus, BrBV), as well as two viruses from the rosy apple aphid (rosy apple aphid virus, RAAV, and *Dysaphis plantaginea* densovirus, *Dpl*DNV). Although no obvious pathology appears to be associated with BrBV infection in the cabbage aphid, we found a negative correlation between the level of BrBV accumulation in the aphid and parasitoid wasp infestation. Analysis of the accumulation of RAAV and *Dpl*DNV in the rosy apple aphid culture showed that RAAV was present at similar levels in all aphids of the infected cultures. RAAV infection resulted in a significant reduction in insect size, but RAAV had no effect on the aphid reproduction rate. High levels of *Dpl*DNV were present in the culture but only in aphids showing melanization and a significantly reduced reproduction rate. Aphids with high *Dpl*DNV levels also had a greater tendency to produce wings and to colonize neighbouring plants.

Contributed paper. Thursday, 18:00. **233**

Positive-strand RNA viral infections of the red imported fire ant, *Solenopsis invicta*

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An expression library was created and 2,304 clones sequenced from a monogyne colony of *Solenopsis invicta*. The primary intention of the project was to utilize homologous gene identification to facilitate discovery of viruses infecting this ant pest that could potentially be used in pest management. Two viruses were ultimately discovered by the method, *Solenopsis invicta* viruses 1 and 2 (SINV-1 and -2). SINV-1 and -2 are positive strand RNA viruses. The SINV-1 genome is monopartite and dicistronic. SINV-2 is monopartite and polycistronic (4 open reading frames). Both viruses possessed consensus sequences characteristic of the helicase, cysteine protease, and RNA-dependent RNA polymerase sequence motifs of positive-strand RNA viruses. Characterization of each viral genome and the potential for use as control agents are discussed.

AUTHOR INDEX
Abstract no. 193 indicates oral presentation; B-12 indicates poster presentation

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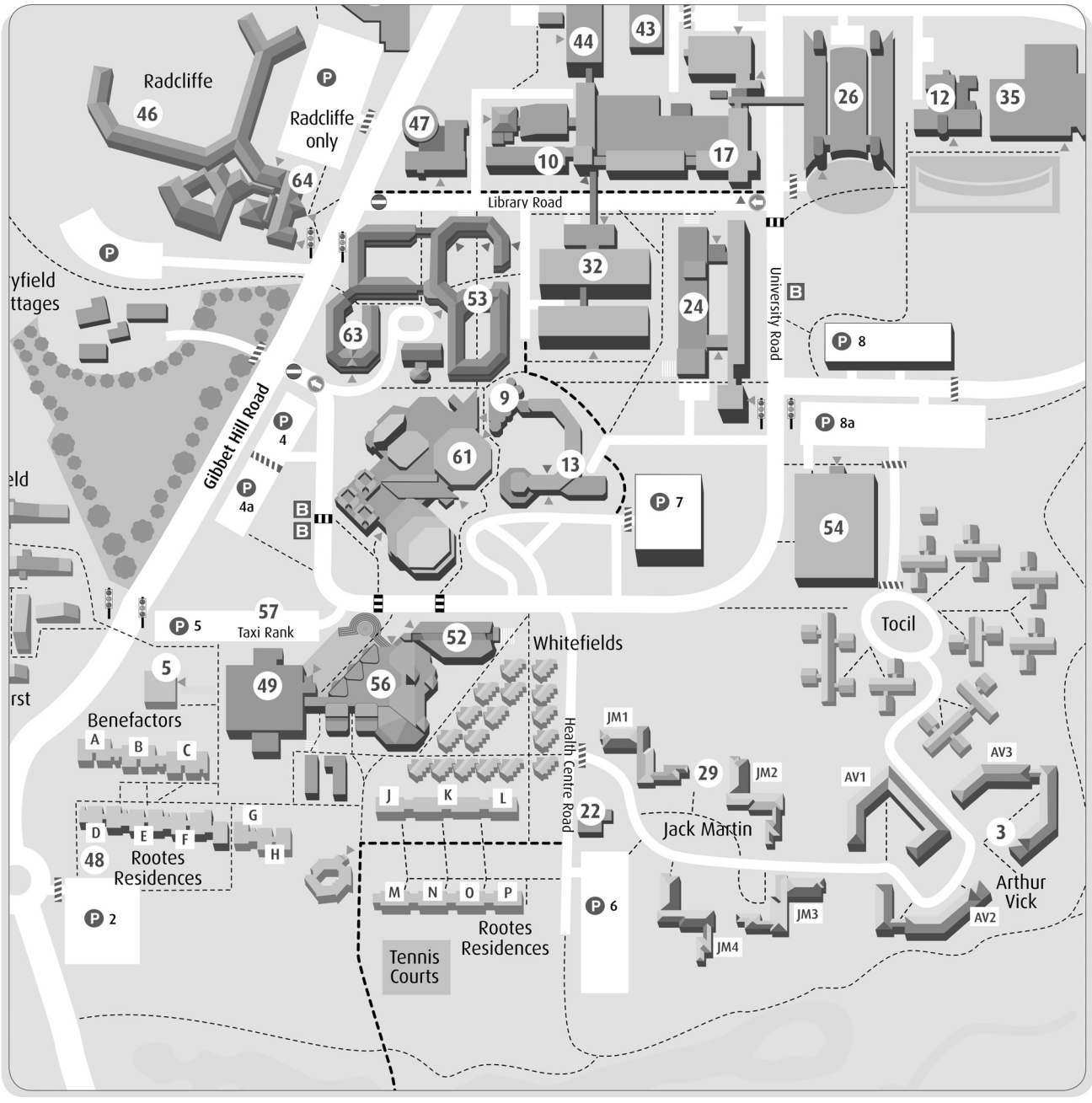
Abstract no. 114 indicates oral presentation; B-34 indicates poster presentation



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Coaches for the Optional Excursion at 13:30 on Tuesday, and for the Banquet and Awards Ceremony at 19:00 on Thursday will leave from the Rootes bus stop, which is in front of the Rootes Social Building (49).

The 5K run/walk at 6:45 on Tuesday will begin at the Cryfield Sports Pavilion, which is a short walk west of Rootes Residences (48).

The BBQ at 19:00 on Tuesday will be held at the Cryfield Sports Pavillion, a short walk west of Rootes Residences (48).

The support of the following organizations for the
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