41\textsuperscript{th} ANNUAL MEETING
of the
Society for
INVERTEBRATE
PATHOLOGY
and
9\textsuperscript{TH} INTERNATIONAL CONFERENCE ON
\textit{BACILLUS THURINGIENSIS}
Incorporating COST862 Action: Bacterial
Toxins for Insect Control

PROGRAM and ABSTRACTS

3-7 August 2008
University of Warwick,
Coventry, UK
2008 SIP Meeting
Meeting at a Glance

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
* Nematodes 1 SS021
16:00 – 16:30 Break Arts Centre Gallery
16:30 – 18:30 Symposium:
* Utilizing Insect 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

TUESDAY— 5 August

6:45 – 8:00 5K Fun Run/Walk Warwick Univ. campus
8:00 – 10:00 Symposium:
* Virulence Factors in Fungal 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.

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### WEDNESDAY— 6 August

<table>
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<th>Time</th>
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| 8:00 – 10:00 | **Symposium:**  
* Entomopathogenic Bacteria Other than *Bacillus*  
* Microsporidia of Aquatic Arthropods  
* Fungi 2  
* Viruses 3 | Arts Centre Theatre SS020 Arts Centre Conf Rm |
| 8:00 – 10:00 | **Contributed Papers:**  
* Fungi 2  
* Viruses 3 | SS021 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  
* Microbial Control 2  
* Viruses 4 | Arts Centre Theatre SS021 Arts Centre Conf Rm |
| 12:00 – 14:00 | **Student Workshop**  
* Spreading the Word: (Lunch provided) Skills for Communicating Science and Getting it Funded | Chancellors Suite, Rootes |
| 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  
* Nematodes 3  
* Viruses 5 | Arts Centre Theatre SS020 Arts Centre Conf Rm |
| 16:00 – 16:30 | Break | Arts Centre Gallery |
| 16:30 – 18:30 | **POSTERS 2**  
* Microbial Control  
* Microsporidia  
* Nematodes  
* Other  
* Viruses | Arts Centre Gallery |
| 17:00 – 18:30 | **COST meeting** | SS020 |
| 18:30 – 19:30 | **Dinner meeting** | 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

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| 8:00 – 10:00 | **Symposia:**  
* Commercialization and Quality Control of Bacterial Insecticides  
* Comparative Genomics of DNA Viruses | Arts Centre Theatre |
| 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  
* Microbial Control 3 | Arts Centre Conf Rm 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  
* Viruses 6 | Arts Centre Conf Rm SS021 |
| 19:00 | **Coaches leave from Rootes bus stop (in front of Rootes Social Bldg.)** | |
| 19:00 | **BANQUET** | Britannia Royal Court Hotel |
| 19:00 | **AND AWARDS CEREMONY** | |
| 19:00 | **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

Vice President  Mark Goettel  Lethbridge Res Ctr, Agriculture & Agri-Food Canada, P.O. Box 3000,  
Lethbridge, AB, T1J 4B1, Canada  
Phone / Fax: (403) 317-2264 / (403) 382-3156  
Email: goettelm@agr.gc.ca

Treasurer  James Becnel  10708 SW 90th Court, Gainesville, FL 32608, USA  
Phone / Fax: (352) 374-5961 / (352) 374-5966  
Email: James.Becnel@ars.usda.gov

Secretary  Jenny Cory  Algoma University College, 1520 Queen Street East, Sault Ste. Marie,  
Ontario, P6A 2G4, CANADA  
Phone / Fax: (705) 541 5619 / (705) 949 6583  
Email: jenny.cory@algomau.ca

Past President  Just Vlak  Wageningen University, Laboratory of Virology,  
Binnenhaven 11, Wageningen 6709 PD, The Netherlands  
Phone / Fax: +31 3 1 748 3090 / +31 31 748 4820  
Email: just.vlak@wur.nl

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Harry Kaya
James Harper
Jürg Huber

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Brian Federici
Flavio Moscardi
Mark Goettel
Kelli Hoover

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David Onstad (Chair)
Cecilia Schmitt
Just Vlak
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Doreen Winstanley
Hisanori Bando
Albrecht Koppenhöfer
Hisanori Bando
Brian Federici
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Michael Dimock
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Endowment / Financial Support
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Michael Dimock
Jürg Huber

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Dudley Pinnock (Chair)
Max Bergoin
Neil Crickmore
Hu Zhihong
James Becnel

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Elizabeth Davidson (Chair)

Lomer Memorial Award
Paresh Shah (Chair)

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Helen Roy (Chair)
Paresh Shah
Neil Crickmore
Elizabeth Davidson
Robert Anderson
Juan Luis Jurat-Fuentes
Andreas Linde
Trevor Jackson
Kerstin Jung
Yasuhsa Kunimi

Awards & Student Contest
Andreas Linde (Chair)
Bryony Bonning
Nguya K. Maniania

2008 ANNUAL MEETING ORGANIZING COMMITTEE

Chair: David Chandler
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
Robert Possee, Judith Pell, Helen Roy
Gill Prince, Trish Wells, Gary Keane, Sally Hilton
John Danquah, David Carpenter, 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 Routes Conference Desk
18:00–21:00 Mixer Arts Centre Gallery

MONDAY - 4 August

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

Opening Ceremonies and SIP Founders’ Memorial Lecture

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

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

Founders’ Memorial Lecture
Dudley Pimmick, Chair, Founders’ Lecture Committee
Honoree: ANDRÉ PAILOTT
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 vanEngelsdorp1, Pennsylvania State Department of Agriculture, Harrisburg, Pennsylvania, USA

11:00 2 Microsporidial infections in hymenopteran pollinators
Ingemar Fries1, Department of Entomology, Swedish University of Agricultural Sciences, Uppsala, Sweden

11:30 3 Applied beeomics: Molecular studies of honey bee disease and resistance
Jay D. Evans1, 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 Routes 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 Cory1; Judy Myers2;

1 Algoa 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. Pedersen1, University of Sheffield, UK

15:00 7 Host-parasite coevolution under environmental variation
Tom J. Little1, University of Edinburgh, UK

15:30 8 The evolutionary ecology of Bt
Michael B. Bonsall1, Oxford University, UK

MONDAY AM

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 Eilenberg1; Gisbert Zimmermann2; Tariq Butt3; Kerstin Jung4; Charlotte Nielsen1; Hermann Strasser1; Milton Typas1, University of Copenhagen, Denmark; 2BBA, Institute for Biological Control, Darmstadt, Germany; 3University of Swansea, Wales, UK; 4University of Innsbruck, Austria; 5University of Athens, Greece

14:15 10 A novel approach to develop biopesticides based on entomopathogenic fungi
Kim, Jae Su1; Woo Eun Ok2; Park Jong Sung3; Kim Yun Sung4; Kim Tae-Joon5; Kim Kyung-Sung1; Roh Jong Yul1; Choi Jae Young5; Je Yeon Ho3; 1AgroLife Research Institute, Dongbu HiTek Co. Ltd., Korea; 2Seoul National University, Korea

14:30 11 STU Host plant effects on fitness of the mating pathogenic fungus Neoseiulus floridana Vitalis W. Wekesa1; Stefania Vital1; Renan A. Silva1; Italo Delalibera Jr.1; 1University of São Paulo, Brazil

14:45 12 Intraguild interactions involving Pandor a neoaphidis at the population scale
Jason Baverstock1; Judith K. Pell2; 1Rothamsted Research, Harpenden, Hertfordshire, UK

15:00 13 STU Enhanced transmission of Pandor a neoaphidis by the invasive ladybird Harmonia axyridis
Patricia M. Wells1, Jason Baverstock1; Michael E.N. Majerus2; Helen E. Roy2; Judith K. Pell1, Rothamsted Research, Harpenden, Hertfordshire, UK; 2University of Cambridge, UK; 1Centre 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/S8-Su strain against medfly Ceratitis capitata (Diptera; Tephritidae) adults Almudena Ortiz-Urquina; Ana Borrego1; Cándido Santiago-Alvarez; Enrique Quezada-Moraga1, University of Córdoba, Spain

15:30 15 Viability of formulations of Beauveria bassiana for use in grain stores
Bransy Taylor1; Belinda Luke1; 1CABI, Bakeham Lane, Egham, Surrey, UK

Symposium (Cross Divisional) 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 Hoch1; Axel Schopf1, 1BOKU 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* Guérin-Méneville Jeffrey Le\(^1\); Susan Bjornson\(^1\), \(^1\)Saint Mary's University, Halifax, Canada

14:45 19 STU Life cycle of a microsporidian isolate (*Noesma* sp.) from the three spot grass yellow butterfly, *Eurema bland arsakia* Yi-chun Tsai\(^1\), Chung-Hsiung Wang\(^2\), \(^1\)National Taiwan University, Taiwan (R.O.C.)

15:00 20 STU A new species, *Vairorhorma ocinariae* n. sp., isolated from *Ocicara lida* Moore (Lepidoptera: Pombycidae) in Taiwan Chih-Yuan Wang\(^1\), Wei-Fong Huang\(^1\), Yi-Chun Tsai\(^1\), Chung-Hsiung Wang\(^2\), \(^1\)National Taiwan University, Taiwan (R.O.C.)

15:15 21 A new microsporidian species isolated from the freshwater shrimp, *Cardina formosa* Tai-Chun Wang\(^1\); Chih-Yuan Wang\(^2\), Wei-Fone Huang\(^2\), Chung-Hsiung Wang\(^2\), \(^1\)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\(^2\); Yi-Ting Yang\(^2\); Chung-Hsiung Wang\(^2\); \(^1\)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 Yuni S. Tokarev\(^1\); Julia M. Malysh\(^2\); Philippe Audiot\(^3\); Igor V. Senderskiy\(^1\); Andrei N. Frolov\(^1\); Sergine Ponsard\(^4\); Denis Bourguet\(^4\); \(^1\)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

16:00-16:30 BREAK

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**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

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**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\(^1\); Chun-karn Boonchoy\(^1\); Julien Lescar\(^1\), \(^1\)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 Moonsoo\(^1\); Urai Chaisin\(^2\); 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 Kamonnut Singkhamaan\(^1\); Boonhiang Promdonkon\(^1\); 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* Suweerawa Limpanawan\(^1\); Panadda Boonserm\(^1\); Boonhiang Promdonkon\(^1\), \(^1\)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 Singkhamaan\(^1\); Panadda Boonserm\(^1\); Boonhiang Promdonkon\(^1\), \(^1\)Mahidol University, Thailand; \(^2\)National Science and Technology Development Agency, Thailand

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**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\(^2\); Yolanda Flores-Lara\(^2\); \(^1\)University of Arizona, Tucson AZ USA

14:15 25 Biochemical and molecular characterization of symbiotic microorganisms from four *Steinernema* from Costa Rica, *S. costaricensis* n.sp. (*CR9*), *S. punctatennis* n. sp. (*L16*), *S. websteri* (*CR5*) and *Steinernema* sp. (*T4*) Lorena Uribe-Lorio\(^1\); S. Patricia Stock\(^2\); Diego Navarro\(^2\); Elena Castillo\(^2\); Maríael Moral\(^2\); \(^1\)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

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

14:00 24 STU Exploring tritrophic interactions: Biological control of an obligate pest by its obligate parasite Keith G. Davies, Rothamsted Research, Harpenden, Hertfordshire, UK
Monday PM

17:30

**39 STU** Loop2 in Cry4Aa domain II, but not loops 1 and 3, is essential for the mosquitoicidal activity against *Culex pipiens* Mohammad Tofazzal Hossain Howlader1; Yusahiro Kagawa2; Hiroshi Sakai3; Tohru Hayakawa1; Okayama University, Okayama, Japan

18:00

**40 STU** Identification of the midgut binding-molecule for Cry4Ba toxin in *Anopheles albimanus* larvae Maria Teresa Fernandez-Luna1; Alejandra Bravo2; Humberto Lanz3; Sarjeet Gill4; Maro Soberon5; Juan Miranda-Rios1; 1National Autonomous University of Mexico, Morelos, Mexico; 2Instituto Nacional de Salud Publica, Morelos, Mexico; 3University of California, Riverside, USA; 4University of Colombia.

18:15

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

### Contributed Papers

**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. Hernioux1; Jennifer S. Cory2; Timothy G. Barraclough3; 1Imperial College London, Ascot, Berkshire, UK; 2Algonia University College, Sault Ste. Marie, Ontario, Canada

16:45

**43** Genome sequence of the complete genome of *Spodoptera frugiperda* multiple nucleopolyhedrovirus isolate from Nicaragua Oihane Simon1; Delia Muñoz2; Trevor Williams3; Primitivo Caballero4; Miguel López-Ferber5; 1Universidad Pública de Navarra, Navarra, Spain; 2Instituto de Ecología AC, Veracruz, Mexico; 3ECole des Mines d’Alès, Alès, France

17:00

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

17:15

**45** Comparative genomics of different isolates of *Cydia pomonella* granulovirus (CpGV) Karolin E. Eberle1; Doreen Winstanley1; Mohammadreza Rezapanah2; Johannes A. Jehle3; 1Agricultural Service Center Palatinate (DLR), Germany; 2Warwick Horticulture Research International, University of Warwick, Wellesbourne, UK; 3Insect Biocontrol Research Department, Tehran, Iran

17:30

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

17:45

**47** Sequence and organization of the *Orgyia leucostigma* nucleopolyhedrovirus genome Renée Lapointe1; Robert J.M. Eveleigh2; Robert J. Graham3; Hillary A.M. Lauzon3; Lilian Pavlik2; Basil M. Arif2; Christopher J. Lucarotti4; 1Sylvan Technologies Inc., Fredericton, New Brunswick, Canada; 2CSIRO Entomology, Canberra, Australia; 3Natural Resources Canada, Canadian Forest Service – Great Lakes Forestry Centre, Ontario, Canada; 4Natural 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 palpalis* salivary gland hypertrophy virus (GpSGHV) from Uganda and Ethiopia Adly M.M. Abd-Alla1; François Cousserans2; Andrew Parker3; Alan Robinson4; Max Bergoin5; 1International Atomic Energy Agency, Vienna, Austria; 2Université Montpellier II, France

18:15

**49** Comparative analysis of viruses that cause salivary gland hypertrophy in *Glossina palpalis* (GpSGHV) and *Musca domestica* (MdSGHV) Alejandra Garcia-Marruniak1; Adly M. M. Abd-Alla2; Andrew G. Parker3; Tamer Z. Salem3; Verena-Ulrike Lietze4; Monique M. van Oers5; James E. Marruniak1; François Cousserans6; Alan S. Robinson7; Just M. Vlak8; Max Bergoin9; Drion G. Boucias1; 1University of Florida, Gainesville, FL USA; 2FAO/IAEA Seibersdorf, Austria; 3 Wageningen University, The Netherlands; 4Université Montpellier II, France

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**DINNER**

**18:30–19:30** Rootes Restaurant

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**SIP Division Business Meetings:**

- **Nematode**
  - Monday evening
  - **Viruses**
    - (19:00–20:00) SS017
  - **Bacteria**
    - (19:00–20:00) SS021
  - **Fungi**
    - (19:30–20:30) SS020
  - **Microsporidia**
    - (19:30–20:30) SS009

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**Nematode-Bacterium Associations Workshop**

- **Monday, 20:00–21:30. SS017**
  - Organiser: Patricia Stock

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**20:00**

**50** Nematode-Bacteria Symbiosis Research Network: Intertwinning knowledge and research tools S. Patricia Stock1; 1University of Arizona, USA

**20:15**

**51** Evolution and genetics of *C. elegans-pathogen* interactions Hinrich Schulteburg1; 1Westphalian Wilhelms-University, Germany

**20:30**

**52** Innate immunity in nematodes and somaclonal cuticle variation as revealed by *Pastoria penetrans* Keith G. Davies1; 1Rothamsted Research, Harpenden, Hertfordshire, UK

**20:45**

**53** The obligate *Wolbachia* endosymbiont in filarial nematodes provides potential targets for disease intervention Barton E. Slagle1; Bo Wu2; Jeremy Foster1; 1New England Biolabs, Inc., Ipswich MAUSA

**21:00**

**54** *Photorhabdus*: Molecular analyses of pathogenicity and mutualism Catherine A. Eason1; David J. Clarke1; 1University College Cork, Ireland

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**Invertebrate Virus Discovery Workshop**

- **Monday, 20:00–21:30. SS021**
  - Organizers: Peter Krell and James Marruniak

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**20:00**

**55** Hunting for insect pathogens: A genomics approach Wayne B. Hunter1; 1USDA, ARS, Fort Pierce, FL USA
20:30 **56** Discovering nucleopolyhedrovirus and iridescent viruses of *Spodoptera* spp. Trevor Williams1; Oihane Simón2; Gabriel Clavijo2; Delia Muñoz2; Rosa Murillo2; Primitivo Caballero2; Robert D. Possee3; Noe Hernández4; Jorge E. Ibarra5; Miguel López-Ferber5; 1Instituto de Ecología AC, Xalapa, Mexico; 2Universidad Pública de Navarra, Pamplona, Spain; 3CEH-Oxford, UK; 4CINVESTAV-IPN, Mexico; 5LGEI, Ecole des Mines d’Alés, Alés, France

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

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


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

Stephen A. Rehner1; Joseph F. Bischoff2; Richard A. Humber3; USDA-ARS, Beltsville, MD USA; USDA-AFPHS, Beltsville, MD USA; USDA-ARS, Ithaca, NY, USA

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

20:30 57 Steven O’Donnell, New Mexico State University, Las Cruces, NM USA; Ray Cowan, University of Illinois, Urbana-Champaign, IL USA; Daniel De Ley, National Institute for Medical Sciences, Mexico City, Mexico

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**Microsporidia Division Workshop** Monday, 20:30-21:30. SS009

**Use of qPCR to Quantify Microsporidia Infection***

Organizer: David Oi.

20:30 **58** Quantifying developing *The holania solenopsae* infections in the red imported fire ant, *Solenopsis invicta*

Steven Valley1; David Oi2, 1USDA-ARS, Gainesville, FL, USA

21:00 **59** Prevalence and levels of *Nosema ceranae* in healthy and declining honey bee colonies

Yaping Chen1; Jay D. Evans1, 1USDA-ARS Bee Research Lab, Beltsville, MD, USA

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**TUESDAY - 5 August**

6:45 **5K Fun Run / Walk**

Warwick Univ Campus

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**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. Leger1, Chengshu Wang2; Weigu Fang3; Sibhao Wang4; 1University of Maryland, USA; 2Shanghai Institutes for Biological Sciences, PRC

8:24 **61** Attenuation of virulence in entomogenous fungi Tariq M. Butt1; Farooq A. Shah1; 1Swansea University, UK

8:48 **62** Developing insect models to study the evolution of fungal pathogens Michael J. Bidochka1; 1Brock University, Canada

9:12 **63** Investigating the biology of plant infection by the rice blast fungus *Mag naporthe grisea* Martin J. Frang1; Michael J. Kershaw1; Diane O. Saunders2; Elise Lambeth2; Ana-lilia Martinez-Rocha1; Nicholas J. Talbot1; 1University of Exeter, UK; 2Universidad de Córdoba, Córdoba, Spain

9:36 **64** Are there overlaps between virulence factors of fungal pathogens of arthropods, plants, and vertebrates? Alice C.L. Churchill1; 1Cornell University, Ithaca, NY, USA

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**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. *kariyaki* delta-endotoxins for the control of the lepidopteran olive tree pathogenic insect *Prays oleae*: From gene cloning to application in the field Samir Jaoua1; Souad Roui1; Slim Tounsi1; Nabil Zouari1; Imène Saadaoui1; Hichem Azzouz1; Lobna Abdelkafi Mesrati1; 1Centre of Biotechnology of Sfax, Tunisia

8:15 **65a** The influence of *Bacillus thuringiensis* on baculovirus transmission dynamics in the cabbage moth, *Mamestra brassicae* Helen Hesketh1; Rosemary S. Hails1; 1NERC Centre for Ecology and Hydrology, Oxford, UK

8:30 **66** STU Effectiveness of *Bt* chickepea and the entomopathogenic fungus *Metarhizium anisopliae* to control *Helicoverpa armigera* (Lepidoptera: Noctuidae)

Nora C. Lawo1; Rod J. Mahon2; Richard J. Milner3; Bidyut K. Sarma4; Thomas J.V. Higgins5; Jörg Romesi6; 1Agroscope Reckenholz-Tänikon Research Station ART, Zurich, Switzerland; 2CSIRO Entomology, Canberra, Australia; 3Assam Agricultural University, Jorhat India; 4CSIRO 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. Franklin1; Judith H. Myers1; 1University of British Columbia, Vancouver, BC, Canada

9:00 **68** Effects of *Diacribroica*-resistant *Cry3Bb1*-Bt maize on saprophagous Diptera and their coleopteran predators Wolfgang Buchel1; Oliver Schlein2; Sabine Prescher2; 1Federal Research Centre for Cultivated Plants, Germany

9:15 **69** Preliminary results on the interaction between *Bacillus thuringiensis* and Red Palm Weevil

Barbara Manachini1; Valentina Mansueti1; Vincenzo Arizzi1; Nicolò Parrinello1; 1University 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 Kalvehi1; Y. Nakamura2; K. Mita2; H. Noda2; R. Ichiki4; S. Nakamura3; K. Kadono-Onda3; 1National Institute of Agrobiological Sciences, Tsukuba, Japan; 2Japan International Research Center for Agricultural Sciences, Tsukuba, Japan

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**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. Koppenhöfer1; Eugene M. Fuzy2; 1Rutgers 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 Rouss2; Sergei Spiridonov2; Elena Ivanova2; Jeremy Pearce2; Paul Severns2; Graeme Nicol1; Michael Wilson1; 1University of Aberdeen, UK; 2Russian Academy of Sciences, Moscow, Russia; 3Becker Underwood, Littlehampton, West Sussex, UK; 4Oregon 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-Vojinovic1; Heikki M.T. Hokkanen1,2; University of Helsinki, Finland

9:00 75 Formulation and application of entomopathogenic nematode infected cadavers for control of Hoplia philanthus in turf Hussain Abid1; Ansari A. Minshad1; Moens Maurice2, Sardar Vallabh Bhai Patel University of Agriculture & Technology, India; Swinsea University, UK; Institute for Agriculture and Fisheries Research, Merelbeke, Belgium

9:15 76 Managing chickpea pod borer, Helicoverpa armigera (Hübner) with Heterorhabditis indica: A success story Prabhjot Acalinar1; B K Patil1; S S Girish2; Shivaleela Shivaleela1;3 University of Agricultural Sciences, Dharwad, Raichur, India

9:30 77 Potential for biocontrol of Diaprepes abbreviatus larvae in nurseries in southern California Kenneth O. Spence1; Edwin E. Lewis1; Jim Bethke1; University of California-Davis, USA; UCCE-San Diego County, San Marcos, CA, USA

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

8:00 78 Functional studies of per os infectivity factors of Helicoverpa armigera single nucleopolyhedrovirus Jingjiao Song1; Ranran Wang1; Fei Deng1; Hualin Wang1; Zhihong Hu1; Wuhan Institute of Virology, Chinese Academy of Sciences, P. R. China

8:15 79 STU Influence of pif and pif2 genes in the dynamics of recombinant insect virus populations Gabriel Clavijo1; Oihane Simon1; Delia Muñoz1; Martine Cerut1; Trevor Williams1; Primitivo Caballero1; Miguel Lopez-Ferber4; Universidad Publica de Navarra, Spain; CNRS, Saint Christol-Les-Alès, France; Instituto de Ecología AC, Xalapa, Veracruz, Mexico; Ecole des Mines d’Ales, Ales, France

8:30 80 AcMNPV ac143 (ode+18), a core gene that forms a cluster with ac142, is essential and mediates BV production Christina B. McCarthy1; Cam Donly1; David A. Theilmann1; Agriculture and Agri-Food, Summerland, BC, Canada; 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 VP90) and itself in Autographa californica multiple nucleopolyhedrovirus Wenbi Wu1; Hanquan Liang1; Chao Liu1; Meijing Yuan1; Kai Yang1; Yi Pang1; 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 Li1; Gary W. Blissard1; 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 Wang1; Ying Tan1; Feifei Yin1; Fei Deng1; Zhihong Hu1; Just M. Vlak2; Hualin Wang1; Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, China; Wageningen University, The Netherlands

9:30 84 STU A highly conserved baculovirus gene p48 is essential for BV production and ODV envelopment Meijin Yuan1; Wenbi Wu1; Chao Liu1; Yanjie Wang1; Chaoyang Hu1; Kai Yang1; Yi Pang1; 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. Carpenter1; Caroline M. Griffiths1; Linda A. King3; 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 Sela1;2; Eyal Maori1; Nitzan Paldi1; Etan Glick1; The Hebrew University of Jerusalem, Israel; BeeLogics, LLC, Miami, FL, USA

11:00 87 The pitfalls of diagnosis interpretation in honey bee pathology: The case of deformed wing virus (DWV) Laurent Gautheir1; Julie Fievet1; Diana Tenti Cheva1; Marc Edouard Colin1; Max Bergoin1; SupAgro Montpellier, Montpellier, France; Université Montpellier 2, Montpellier, France

11:30 88 Transmission and pathogenesis of DWV Sebastian Gisder1; Constanze Yue1; Elke Genersch1; Institute for Bee Research, Germany

12:00 89 Host specificity of honey bee viruses and transmission routes—Implications for pollinator health Rajwinder Singh1; Abby Kalkstein1; Edwin Rajotte1; Dennis vanEngelsdorp1; Nancy Ostiguy1; Eddie Holmes2; Claude dePamphilis3; Rick Donvall1; Ian Lipkin1; Diana Cox-Foster1; Dept of Entomology, and Dept of Biology, Penn State University, University Park, PA, USA; Pennsylvania Dept of Agriculture, Harrisburg, PA, USA; Mailman School of Public Health, Columbia University, USA

POSTERS – 1 Tuesday, 10:30-12:30. Arts Centre Gallery

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 Pauchet1; Heiko Vogel2; Paul Wilkinson1; David G. Heckel1; Richard H. ffrench-Constant1; University of Exeter in Cornwall, Penryn, UK; Max Planck Institute for Chemical Ecology, Germany

B-02 Understanding the interactions of two novel Cyt-toxins Kara S. Giddings1; Andrew M. Wollacott1; Monsanto Company, Chesterfield MO, USA; Monsanto Company, Cambridge MA, USA

B-03 Variability in the cadherin gene in the European corn borer, Ostrinia nubilalis (Hübner) Yolanda Bel1; Juan Ferré2; Baltasar Escriche1; University of Valencia, Spain

B-04 Comparison of wild-type and mutant forms of Bt toxin Cyt1A in molecular dynamics simulations Xiaochuan Li1; Dexuan Xie1; Peter Butko1; Boston University, Boston, MA USA; University of Wisconsin, Milwaukee, WI, USA; University of Maryland, Baltimore, MD, USA
B-05 Chitinase profiles and insecticidal effects of bacteria originated from hazelnut pests Zihni Demirbag; Bahar A. Adem; Kazim Sezen; Remziye Nalcacioglu; Karadeniz Technical University, Trabzon, Turkey

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

B-07 Expression profiles of aminopeptidase genes in Heliothis virescens larvae exposed to Bt toxins Omathagne P. Perera; Anais S. Castagnola; Juan Luis Jurat-Puentes; Craig A. Abel; USDA-ARS, Stoneville, MS, USA; 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; Gloria Navarro-Cerrillo; Ruud A. de Maagd; Baltasar Escrì; Salvador Herrero; Universitat de Valencia, Spain; Business Unit Bioscience Plant Research International B.V., Wageningen, The Netherlands

B-09 Drosophila embryos as a novel system to test insecticidal toxins in vivo Andrea J. Dowling; Isabella Vlisidou; Nicholas R. Waterfield; Richard H. French-Constant; William Wood; University of Exeter in Cornwall, Falmouth, UK; University of Bath, UK

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

B-11 STU Characterization of the Heliothis virescens midgut regenerative response upon treatment with Bacillus thuringiensis Cry1Ac toxin Anais S. Castagnola; Omathagne P. Perera; Juan Luis Jurat-Puentes; University of Tennessee, Knoxville, TN, USA; 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; Neil Crickmore; University of Sussex, Falmer, Brighton, UK

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

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

B-15 STU The efficacy of non-mosquitoidal Malaysian Bt isolates (Bt18) against three leucine kinase lines (CEM-SS, CCRF-SB and CCRF-HSB-2) and its mode of cell death Chan K. Keong; Nadarajah V. Devi; Mohamed S. Mariam; Abdullah Mahu; International Medical University, Kuala Lumpur, Malaysia; 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; Ana Rodrigo-Simón; Juan Ferré; Universitat de València, Spain

B-18 STU Study of two midgut aminopeptidases from Ostrinia nubilalis Hübner Cristina M. Crava; Yolanda Bel; Barbara Manachini; Baltasar Escrì; Universitat de València, València, Spain; 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; Bryony C. Bonning; Iowa State University, Ames IA, USA

B-20 STU Analysis of receptor-binding region for effective improvement of Cry1Aa insecticidal activity Fumiaki Obata; Madoka Kitani; Yukino Inoue; Takuya Kotsani; Yoko Harashima; Chintatsu Morimoto; Yasushi Hoshino; Delwar M. Hossain; Ryochi Sato; Tokyo University of Agriculture and Technology, Koganei, Tokyo, Japan

B-21 Genetic stability of the putative marker Bacillus thuringiensis S76GF expressing a green fluorescence protein (GFP) in the absence of selective pressure Juliana C. de Orem; Ana F. Parente; Mariana T R Lira; Tayana Kariya; Isabela M M de Oliveira; Marlene T. De-Souza; Brasilia University, Brazil

B-22 STU Construction of modified Bacillus thuringiensis cry1Ac genes based on cry1-S genes through multi-site-directed mutagenesis Hong Guang Xu; Jong Yul Roh; Jae Young Choi; Hee Jin Shin; Yong Wang; Qin Liu; Soo Dong Woo; Byung Rae Jin; Yeon Ho Je; Seoul National University, Korea; Research Institute for Agriculture and Life Sciences, Seoul National University, Korea; Chungbuk National University, Korea; 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; Jie Zhang; Changlong Shu; Fuping Song; Guixin Yan; Dafang Huang; Northeast Agricultural University, Harbin, China; Chinese Academy of Agricultural Sciences, Beijing, China

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

B-25 Studies on protease-resistant core form of Bacillus thuringiensis Cry1Ia toxin Shuyuan Guo; Yancai Zhang; Jie Zhang; Fuping Song; Dafang Huang; Beijing Institute of Technology, China; CAAS, Beijing, China; Chinese Academy of Agricultural Sciences, Beijing, China

B-26 STU 20kb DNA: What is it doing in Bt crystals? Seher Fazal; Christopher Jones; Neil Crickmore; 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; Mariam Dammak; Samir Jaoua; Centre of Biotechnology of Sfax, Sfax, Tunisia

B-28 Bacillus thuringiensis serovar thompsoni HD542 crystal proteins: Solubilization, activation, and insecticidal activity Samir Naimov; Rumyana Boncheva; Ruud deMaagd; University of Plovdiv "Paisii Hilendarski", Bulgaria; 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 Świeciecki1; Dennis K. Bideshi2; Magdalena Czajkowska; Sylvia Kotowicz1; University of
Bialystok, Poland; 1University of California, Riverside, California USA

B-30 Characterization of a novel Cry9Bb δ-endotoxin from Bacillus thuringiensis Joseídse O. Silva-Wernecke; David J.
Eiller1; Embrapa Recursos Genéticos e Biotecnologia, Brasília, Brazil; 1University of Cambridge, UK

B-31 STU Identification and cloning of novel cry genes from Bacillus thuringiensis strain Y41 Changdong Shu1; Xudong
Su1; Jie Zhang1; Dafang Huang1; Fuping Song1; Institute of Plant Protection, and 2Biotecnology Research Institute,
Chinese Academy of Agricultural Sciences, Beijing, P. R. China

B-32 STU The characterization of novel Bt toxins Zenas
George1; Neil Crickmore1; 1University 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. Noguera1; Jorge E. Ibarra1; Departamento de Biotecnología y Bioquímica, CINVESTAV-IPN, Irapuato,
Mexico

B-34 Genetic diversity of cry gene sequences of Bacillus thuringiensis strains analyzed by denaturing gradient gel
electrophoresis Corina M. Berón1; Macarena Pérez-Cenci1; Graciela L. Salerno1; 1Fundación para Investigaciones
Biológicas Aplicadas (FIBA), Argentina

B-35 Cyanogenesis in Pseudomonas entomophila: An entomopathogenic bacterium Ben Ryall; Hannah Nasser1;
Dimitris Miossides1; Huw D. Williams1; 1Imperial College London, UK; 1University of Thessaly, Larissa, Greece

B-36 Bacillus thuringiensis: Genetic diversity of Brazilian Lepidoptera specific isolates Ana M. Guidelli-Thuler1;
Janete A. Desidério Sena1; Irlan L. de Abreu1; Camila C. Davolos1; Sergio B. Alves1; Ricardo A. Polanczyk1; Fernando
H. Valicente1; Manoel Victor F. Lemos1; Universidade Estadual Julio Mesquita Filho (UNESP Jaboticabal), Brasil;
1Escola Superior de Agricultura Luiz de Queiroz (ESALQ-USP), Brasil; 2Centro de Ciencias Agrarias - Universidade
Federal do Espírito Santo, Brasil; 3Empresa Brasileira de Pesquisa Agropecuária, Brasil

B-37 STU Characterization of an endophytic Bacillus thuringiensis strain isolated from sugar cane Marise T.
Suzuki1; C. Sara Hernández-Rodríguez1; Welington L. de Araújo1; Juan Ferré1; 1Universidad de València, Spain;
2Universidade 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. Kleespies1; Andreas Leclercque1; 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 subspp. morrisonii strains isolated from Thaumetopoea pityocampa
Den. and Schiff. (Lep., Thaumetopoeidae) Hanice Kat1; Ilkka A. Ince1; Kazim Sezen2; Serife Uç1; Zihni Demirbag2;
1Giresun University, Turkey; 2Karadeniz 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-Quist1; Pedro Castañera1; Joel González-Cabrera1; Instituto
Valenciano de Investigaciones Agrarias, Spain; 1Centro de Investigaciones Biológicas, Madrid, Spain

B-42 Susceptibility to Bacillus thuringiensis of neonates and other larvae of Tortrix viridana L. (Lepidoptera: Torricidae)
from a natural reserve Barbara Manchini1; Filippo Castiglia1; 1University of Palermo, Italy; 2Azienda Regionale Foresti Demaniali, Palermo, Italy

B-43 Bacillus thuringiensis as a biological control agent for the red palm weevil, Rhynchophorus ferrugineus (Oliv.)
(Coleoptera, Curculionidae) Barbara Manchini; Paolo Lo Bue; Elio 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 Guo1; Donghai Peng1; Weiyi Li1; Sisi Ji1; Pengxia
Wang1; Ziniu Yu1; Ming Sun1; 1Huzhong Agricultural University, Wuhan, P.R. China

B-45 Physiological characterization of accumulated poly-β-
hydroxybutyrate(PHB) in Bacillus thuringiensis
Chen Deju1; Yan Jin1; Meng Ying1; Chen Shouwen1; Sun
Ming1; Yu Ziniu1; 1Huzhong Agricultural University, Wuhan P. R. China

B-46 Influence of different strategies of European corn borer
(Ostrinia nubilalis Hübnern) control on the content of contaminants in maize Vladan Fult1; Jitka Stará2; František
Kocourek1; Ludmila Slezáková1; Jana Hajšlová1; Vladimir Kocourek1; O. Lucina1; J. Hontzecák1; Jana Tichá1; Alexandre
Kropková1; Monika Gociéková1; 1Crop Research Institute, Prague, Czech Republic; 2Institute of Chemical Technology
Prague, Czech Republic

B-47 Efficacy of different strategies of European corn borer
(Ostrinia nubilalis Hübnern) control in maize Jitka Stará2;
Vladan Fult1; František Kocourek1; Ludmila Slezáková1;
1Crop Research Institute, Prague, Czech Republic

B-48 Identification of commercial BT-strains by molecular
markers Gian Paolo Barzantini1; Elena Così1; Pietro Runice1;
Pio F. Roveri1; 1C.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 Zemek1; 1Institute 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
(Boisdual) Maria A. Barbutx1; Delia Muñoz1; Ibarra Ruiz,
de Escudero1; 1Instituto Nacional de Investigaciones Agropecuarias, Argentina

B-51 Development of the proteinaceous insecticide from a soil
bacterium (Bacillus thuringiensis) using phage display Delwar M. Hossain1; Takuya Kotani1; Chiharu Morimoto1;
Yuko Harashima1; Ryosichi Satō1; 1Tokyo 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 Venkatesammy Balaubramani1; P. R. Johnston1; Neil
Crickmore1; 1Tamil Nadu Agricultural University, Coimbatore, India; 2University of Sussex, Brighton, UK
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. Avery1; Wayne B. Hunter2; David G. Hall1; Mark A. Jackson1; Michael E. Rogers3; Charles A. Powell1; 1University of Florida, Ft. Pierce, FL, USA; 2USDA, ARS, Ft. Pierce, FL, USA; 3USDA, ARS, Peoria, IL, USA

Potential of topical application, leaf residue and soil drench of fungus Paecilomyces fumosoroseus (Deuteromycotina: Hyphomycetes) for killing citrus weevil: Laboratory and greenhouse investigation

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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Potential of topical application, leaf residue and soil drench of fungus Paecilomyces fumosoroseus (Deuteromycotina: Hyphomycetes) for killing citrus weevil: Laboratory and greenhouse investigation
F-24 Avoidance of entomopathogenic fungi by insect predators
Nicolai V. Meyling1; Emma Ormond2; Helen E. Roy3; Judith K. Pel1; University of Copenhagen, Denmark; 2Anglia Ruskin University, Cambridge, UK; 3NERC Centre for Ecology and Hydrology, Cambridgeshire, UK; 4Rothamsted 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. Fernandes1; Chad A. Keyser2; Draznio E. N. Rangel1; R. Nelson Foster3; Donald W. Roberts1, 1Utah State University, Logan, UT, USA; 2USDA/APHIS/PPQ/CPhST Lab, Phoenix, AZ, USA

F-26 Survey for entomopathogenic fungi from Rhynchophorus ferrugineus (Oliv.) (Coleoptera, Curculionidae) Barba 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 Paparu1; Thomas Dubois2; Daniel Coyne3; Claire Munro4; Alts Vlijmoen1; University of Pretoria, South Africa; 2International Institute of Tropical Agriculture, Kampala, Uganda; 3University 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 Aradottir1, 1University of Iceland, Reykjavik, Iceland; 2Richard Harrington2; Angela Karp3; Steve Hanley3; Ian Shield3; William Macalpine4; Matilda Collins5; Simon Leather6; Judith Peli7; 1Rothamsted Research, Harpenden, Hertfordshire, UK; 2University of Exeter in Cornwall, Penryn, 3University of Bristol, Bristol, UK; 4University of Auckland, Auckland, New Zealand; 5University of Manchester, Manchester, UK; 6University of East Anglia, Norwich, UK; 7University of Exeter, Exeter, 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) Wed., 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 Opeka; Bruno Lemaitre, 1Ecole Polytechnique Federale de Lausanne, Switzerland

8:30 91 Virulence determinants of Yersinia entomophaga MH96: a genomic perspective. Mark R H Hurst1; Regina Shaw2; William G. Farmerie3; Anette Becher2; 1AgResearch, Bioprocessing and Biosecurity, Canterbury, New Zealand; 2University of Florida, Gainesville, FL, USA; 3AgResearch, Invermay, New Zealand

9:00 92 Insecticidal toxins from Photorhabdus: Comparative genomics and Rapid Virulence Annotation (RVA) Richard H. ffrench-Constant1; Stewart Hinchliffe2; Michelle Hares3; Andrea J. Dowling2; Nicholas Waterfield3; Isabella Vlisidou2; Maria Sanchez Contreras4; 1University of Exeter in Cornwall, Penryn, UK; 2University of Bath, UK

9:30 93 Pathogenesis of Serratia entomophila (Enterobacteriaceae) towards the New Zealand grass grub Costelytra zealandica. Trevor A. Jackson1; Sean M. Marshall2; Mark R.H. Hurst3; Drion G. Bouclias4; Heather S. Gatehouse2; John C. Christeller1; 1AgResearch, Canterbury, New Zealand; 2University of Florida, Gainesville, FL, USA; 3Horticulture and Food Research Institute, New Zealand

Symposium (Microsporida 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 Hyliš1; 1Charles University, Prague, Czech Republic

8:20 95 Evolutionary interactions between microsporidia and their hosts: Lessons from an ancient lake Judith E. Smith1; Qui Yang2; Ravid M. Kamalinyov3; Dmitry Y. Sherbakov4; 1Leeds University, UK; 2Siberian Branch of Russian Academy of Sciences, Irkutsk, Russia

8:40 96 Microsporidia in freshwater Amphipods: an overview and an example Remi A. Wattier1; Karolina Bacela1; Thierry Rigaud1; 1Université de Bourgogne, Dijon, Burgundy, France

9:00 97 Coevolutionary dynamics of host-parasite interactions in natural Daphnia populations Ellen Decaestecker1; 1K.U.Leuven - Campus Kortrijk, Belgium

9:20 98 Epizootiological studies of Amblyospora camposi (Microsporidia: Amblyosporidae) in Culex renator (Diptera: Culicidae) and Paracyclops fimbriatus fimbriatus (Copepoda: Cyclopidae) in a bromeliad habitat Victoria Miele1; James J. Becnel2; Gerardo A. Marti3; María C. Tranchida1; Juan J. García1; 1Centro de Estudios Parasitológicos y de Vectores-CEPAVE (UNLP-CONICET), Argentina; 2USDA, ARS, Gainesville, FL, USA

9:40 99 Intracellular microsporidians in crustaceans: The genus Enterospora Grant D. Staintford1; 1Centre for Environment, Fisheries and Aquaculture Science, Weymouth, Dorset, UK

Contributed Papers Wednesday, 8:00-10:00. SS021 FUNGI 2

8:00 100 Genetic analysis of conidiation mutants in Metarhizium anisopliae derived by Agrobacterium-mediated mutagenesis Farah-Jade Dryburgh3; Weiguang Fang1; Raymond J. St. Leger2; Michael J. Bidochka1; 1Brock University, ON, Canada; 2University of Maryland, College Park, Maryland, USA

8:15 101 Directed adaptation of Metarhizium anisopliae to cockroach cuticle Eudes de Crecy1; Nemat O. Keyhani1; 1Evolutec LLC, Gainesville, FL, USA; 2University 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 Gindin1; Dana Ment2; Asael Rot3; Ilamar Glazer1; Michael Samish1; 1The Volcani Center, (ARO), Bet Dagan, Israel; 2Kimron Veterinary Institute, Bet Dagan, Israel
**Symposium (Div. of Nematodes) Wednesday, 10:30-12:30. SS020**

**Entomopathogenic Nematode Application Technology in IPM**

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

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

**BACTERIA 2**

Moderator: David Pauron.

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**103** A proteomic approach to the identification of proteins differentially expressed in the conidia and mycelium of the entomopathogenic fungus *Metarhizium anisopliae* Sérgio H. Silva¹; Bruno H. R. Barros¹; Everaldo R. Marques¹; Ana Patrícia Yatsuda¹; Donald W. Roberts²; Giberto U. L. Braga¹.

¹Universidade de São Paulo, Brazil; ²Utah State University, Logan, UT, USA

**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. Gómez; National Centre of Coffee Research CENICAFE-FNC, Plan Alto, Chinchán, Caldas, Colombia

**105 STU** Alkane degradation by *Beauveria bassiana*: Gene expression analysis of cytochrome P450 monoxygenases Nicolas Pedrini¹,²; Patricia Suárez; Nemat O. Keyhani².

¹Instituto de Investigaciones Bioquímicas de La Plata (CCT CONICET-UNLP), Argentina; ²University of Florida, Gainesville, FL, USA

**106 STU** May *Beauveria bassiana* secreted proteins be virulence factors? Almudena Ortiz-Urquiza¹; Laura Riveiro-Miranda³; Cándido Santiago-Alvarez²; Enrique Quesada-Moraga¹; ¹University of Córdoba, Spain

**107** Live cell imaging of endocytosis and membrane properties of *Beauveria bassiana* in vitro and hemolymph derived cells Michael W. Lewis¹; Ines V. R obalino¹; Nemat O. Keyhani²; ¹University of Florida, Gainesville, FL, USA

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

**VIRUSES 3**

Moderators: Doreen Winstanley and Rollice Clem.

**108** Deletion of the egt gene reduces within-host competitive fitness Mark Zwart¹; Wopke van de Werf³; Monique van Oers¹; Lia Hemerik¹; Jan van der Lent⁴; Arjan G. M. de Visser¹; Just M. Vlak¹; Jenny S. Corz²; Wageningen University, The Netherlands; ³Algoma University College, Sault Ste. Marie, ON, Canada

**109 STU** Characterization of climbing behavior gene in recombinant baculoviruses Matthew R. Gardner¹; James M. Slavicek²; Scott M. Geib¹; Kelli Hoover¹; Pennsylvania State University, USA; ²USDA Forest Service, Delaware, OH, USA

**110 STU** Conservation of DNA photolyase genes in plusuline nucleopolyhedroviruses Fang Xu¹; Just M. Vlak¹; Monique Van Oers¹; Wageningen University, The Netherlands

**111 Chrysodeixis chalcites nucleopolyhedrovirus encodes an active DNA photolyase Monique M. van Oers¹; Margit H. Lampen¹; Monika I. Bajek³; Fang Xu¹; Just M. Vlak¹; André P.M. Eker²; Wageningen University, The Netherlands; ³Erasmus University Medical Centre, Rotterdam, the Netherlands

**112 STU** Anti-viral defenses in gypsy moth larvae: Evidence for the importance of immune responses within the host James R. McNeil¹; Diana Cox-Foster¹; Lauren Ellis¹; Kelli Hoover³; ³Penn State University, University Park, PA, USA

**113 Baculovirus infection of immunosuppressed *S. littoralis* as a tool to study the lepidopteran anti-viral response Nor Chejanovsky; Haddassah Rivkin¹; Itit Ornan¹; ¹Entomology The Volcani Center, Bet Dagan, Israel

**114 An AcNPV gfp knockout mutant exhibits a defect in systemic infection of *Trichoplusia ni* larvae John C. Means¹; A. Lorena Passarelli¹; ¹Kansas State University, Manhattan KS, USA

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**115** Current status in application technology Peters Arne¹; ¹E-Nema, Raisdorf, Germany

**116** Cadaver application Claudia Dolinski¹; Edwin E. Lewis¹; David Shapiro-Ilan¹; ¹Universidade Estadual do Norte Fluminense Darcy Ribeiro, Brazil; ²University of California Davis, CA, USA; ³USDA-ARS, Byron, GA, USA

**117 Above ground and cryptic habitats application Richard Glass¹; Keith F. Walters¹; ¹Central Science Laboratory, Sand Hutton, York, UK

**118** Enhancing post-application survival of entomopathogenic nematodes Terry Lacey¹; ¹USDA-ARS, Yakima Agricultural Research Laboratory, Wapato, WA, USA

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**119** A novel gene cluster encoding an insect toxin in plant-associated strains of *Pseudomonas fluorescens* Maria Pechy-Tarr¹; Denny J. Bruck¹; Monika Maurhofer¹; Esther Fischer¹; Christelle Voge¹; Jurg Grunder¹; Joyce E. Loper¹; Christoph Keel¹; ¹University of Lausanne, Switzerland; ²USDA-ARS Corvallis OR, USA; ³Swiss Federal Institute of Technology, Zurich, Switzerland; ⁴University of Applied Sciences HSW, Switzerland

**120 STU** Functional characterisation of a cell cycle inhibiting factor (CIF) in the entomopathogenic bacteria *Photobahdus* Carolina Varela Chave¹; Grégory Taieb¹; Grégoire Jubelin¹; Gabriel Courties¹; Alain Givaudan¹; Eric Oswald¹; Jean-Michel Escoubas¹; Robert Zumbühl¹; ¹Università Montpellier 2, France; ²UMR, Toulouse, France

**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 *Photobahdus luminescens* Ziad Ali Khattar¹; Anne Lanos¹; Sylvie Pagés¹; Mireille Kallasty¹; Sophie Gaudriault¹; Alain Givaudan¹; ¹Università Montpellier 2, France; ²Università Saint-Joseph, Beirut, Lebanon

**122 STU** Structural studies of toxin complexes Michelle C. Hayes¹; Corinne Smith¹; Sarah Lee¹; Richard H. French-Constant¹; ¹University of Exeter, Penryn, Cornwall, UK; ²University of Warwick, Coventry, UK; ³University of Warwick, Warwick HRI, Wellesbourne, Warwick, UK

**123 STU** Interaction between Cry1Ab oligomer and their receptors alkaline phosphatase and aminopeptidase-N from *Manduca sexta* Iván Arenas¹; Alejandro Bravo¹; Mario Soberón¹; Isabel Gómez¹; ¹Biotechnology Institute, UNAM, Cuernavaca, Morelos, México

**124 STU** Cry1Ab oligomeric structure elucidated by transmission electron microscopy Nuria Jiménez-Juárez¹; Liliana Pardo-Lopez¹; Rosana Sánchez¹; Carlos Muñoz-Garay¹; Christos Savva¹; Andreas Holzenberg¹; Mario Soberón¹; Alejandro Bravo¹; ¹Instituto de Biotecnología UNAM, Morelos, México; ²Texas A&M University, USA
11:30 | 138 STU | Aggregation and infection risk in Lepidoptera
Joanna C. McTigue1; Steve M. Sait2; Rosie S. Hails3; Centre for Ecology and Hydrology, Oxford, UK; 4University of Leeds, Leeds, UK
11:45 | 139 STU | Resistance to the CpGV: Improved efficiency by selection pressure on resistant hosts Marie Berling1; Miguel Lopez-Ferber1; Christine Blachère-Lopez2; Benoît Sauphanor3; Antoine Bonhomme3; 4EMA - centre LGEI, Ales, France; 5INRA, Avignon, France; 6NP (Arysta LifeScience), Pau, France
12:00 | 140 Real-time PCR analysis of a mixed infection of granulovirus and nucleopolyhedrovirus from Adoxophyes orana Sally H. Hilton1; Gary Keane2; Doreen Winstanley1; 1Warwick 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 Clavijo1; Oihane Simon2; Delia Muñoz3; Trevor Williams4; Miguel Lopez-Ferber5; Primitivo Caballero6; 1Universidad Publica de Navarra, Spain; 2Instituto de Ecología AC, Veracruz Mexico; 3Ecole des Mines d’Alès, France

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**Student Workshop**

Wednesday, 12:00-14:00. Chancellors, Roots

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

Organizers/Moderators: Onya Opota and Patricia Stock.

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12:00 | 142 Delivering oral presentations Brian A. Federici1; 1University of California-Riverside, Riverside, CA, USA
12:30 | 143 Editing and reviewing scientific manuscripts John D. Vandenberg1; 1USDA-ARS, Ithaca, New York, USA
13:00 | 144 Strategies for writing successful grant applications Peter J. Krell1; 1University of Guelph, Canada
13:30 | 145 What funding agencies want: Tips for getting your research funded S. Patricia Stock1; 1University of Arizona, USA

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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 Yue1; Anne Fünfhais1; Ainura Ashiratilova1; Elke Genersch1; 1Institute for Bee Research, Hohen Neuendorf, Germany
14:45 **148** Sexual transmission of deformed wing virus in honeybees Joachim R. de Miranda; Ingeram Fries; Queen's University Belfast, Northern Ireland; 2Swedish University of Agricultural Sciences, Uppsala, Sweden

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

15:30 **150** Co-evolution of mites and social honeybees in Asia Denis L. Anderson; CSIRO Entomology, Canberra, Australia

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**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; Carl Nystrom; 1Canadian 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; Michael B. Bonsall; 1Oxford University, Oxford, UK

14:30 **153** Pathogenesis of *Bacillus thuringiensis* subsp. kurstaki in spruce budworm and gypsy moth Kees van Frankenhuyzen; Yuehong Liu; 1Natural 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; Terry D. Ericsson; 1Simon Fraser University, Burnaby, BC, Canada; 2University of the Fraser Valley, Abbotsford, BC, Canada; 3University of British Columbia, Vancouver, BC, Canada

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

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

15:30 **157** STU The ger genes of *P. thornei* are responsible for the alkaline-activation of germination in *Bacillus thuringiensis* subsp. *Israelensis* Mostafa Abdoorrahem; Colin Berry; 1Cardiff 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; William J. Moar; 1Auburn University, AL, USA

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**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; Chia-Hung Wang; Wen-Kai Hsiao; 1Yu-Chan Chao; 2Institute 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; Rollie J. Clem; 1Kansas State University, Manhattan KS, USA

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

14:45 **168** AcMNPV late expression factor 3 (LEF-3) functional domains for their role in nucleic localization and baculovirus DNA replication Victoria Au; 1Eric B. Carstens; 2Queen'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 Loganuda Giri; Huorang Li; David Sandgren; David W. Murhammer; Bryony C. Bonning; Mike Feiss; Richard Roller; 1University of Iowa, Iowa City, IA, USA; 2Iowa State University, Ames, IA, USA

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**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; Gulumser Cobanoglu; Ed E. Lewis; Harry K. Kaya; 1Kumiai Chemical Industry Co, Shizuoka, Japan; 2Hacettepe University, Ankara, Turkey; 3University of California, Davis, CA, USA

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

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

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

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

15:15 **164** Hunter to be hunted: Predator mites and entomopathogenic nematodes Mehmet Karagoz; Selcuk Hazir; Ibrahim Cakmak; Baris Gulcu; Harry K. Kaya; 1University of Adnan Menderes, Aydin, Turkey; 2University of Adnan Menderes, Aydin, Turkey; 3University of California, Davis, CA, USA
15:15 170 STU Structural and functional analysis of the Chilo iridescent virus DNA polymerase promoter Ikbal Agah Ince1, 2; Renziye Nałacigło1; Zihni Demirbaş2; Just M. Vilak3; Monique M. van Oers1; Wageningen UR, The Netherlands; 1Karadeniz Technical University, Trabzon, Turkey

15:30 171 STU Suppression of AcMNPV gene expression in mammalian cells Ryosuke Fujita1; Shinichiro Asano1; Ken Sahara1; Hisanori Bando1; Hokkaido University, Japan

15:45 172 SV40 polyadenylation (pA) signal increases transcription but reduces protein production in baculovirus expression vector system Craig P. Seaborn1; Tamer Z. Salem1; Colin M. Turner1; Huitl Xue1; Xiao-Wen Cheng1; 1Miami University, Oxford, Ohio, USA

16:00–16:30 BREAK Arts Centre Gallery

POSTERS ~ 2

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

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. iberiaeisen based formulations in Vietnam Binh D. Ngo1; Tuan D. Nguyen1; Ha T. Trinh1; Vietnamese Academy of Science and Technology, Hanoi, Vietnam

MC-01 Comparison of phytopathogenic antagonism between Bacillus subtilis and Bacillus thuringiensis strains transformed with Cha gene from Serratia marcescens ATCC990 Feng-Chia Hsieh1; Jui-Tang Tseng1; Suey-Sheng Kao1; 1Taiwan 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 Yal Rohl1; Jae Young Choi1; Yong Wang1; Hee Jin Shim1; Jin Li1; Hong Guan Xu1; Jin Cheol Kim1; Yeon Ho Je1; 1Seoul National University, Korea; 2Korea Research Institute of Chemical Technology, Daejeon, Korea

MC-03 Screening of Bacillus thuringiensis to the two-spotted spider mite Tetramychus urticae Ricardo A. Polanczyk1; Dirceu Prattisoli1; Luiz Flávio V. Silveira1; Cláudio R. Franco1; Julieler G. Cocho1t; Launa P. de Souza1; Eduardo D. Grecco1; 1Centro de Ciências Agrárias - Universidade Federal do Espírito Santo, Brasil

MC-04 Selection of Bacillus thuringiensis Cry toxins for the control of Spilothrips oryzae (Coleoptera: Curculionidae) Najara da Silva1; Manoel Victor F. Lemos1; Ricardo A. Polanczyk2; Ana Maria G. Thuler2; Irlan L. de Abreu1; Camila C. Davolos1; Sergio B. Alves1; 1Universidade Estadual Júlio Mesquita Filho (UNESP) Jabori, Brasil; 2Centro de Ciências Agrárias - Universidade Federal do Espírito Santo, Brasil; 3Escola Superior de Agricultura Luís de Queiroz (ESALQ/USP), Brasil

MC-05 Susceptibility of Trichopusus ni (Lepidoptera: Noctuidae) to Bacillus thuringiensis Ricardo A. Polanczyk1; Eduardo D. Grecco1; Dirceu Prattisoli1; Cláudio R. Franco1; Luiz Flávio V. Silveira1; 1Centro 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. Bargas1; Aleksandra Beral1; Delia Muñoz2; Iligo Ruiz de Escudero1; Primitivo Caballero1; 1Universidad 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 Lāga Jankevica1; Rita Seskena1; Agnis Smits2; Ivars Zarins2; 1University of Latvia, Latvia; 2Latvian State Forest Research Institute "Silava", Latvia

MC-08 Searching for pathogens to control stored product mites (Acari: Acaridida). Jan Hubert1; Tomas Erban, Crop Research Institute, Prague, Czechia

MC-09 Microbial control of insect pests in temperate orchard systems: Status and future prospects David Shapiro-Ilan1; Lawrence A. Lacey1; 1USDA-ARS, Byron, GA, USA; 2USDA-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. Chkhubianiashvili1; I. Malania1; M. Kakhadze1; N. Mikaia1; Kanchaveli L. Institute of Plant Protection, Tbilisi, Georgia

MC-11 Potential for entomopathogens against invasive species in landscape ornamentals in Florida Steven P. Arthurs1; Lance Osborne1; 1Mid-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 Pedrin1; Carolina Cambiazzo1; Patricia Juarez1; 1Instituto de Investigaciones Bioquímicas de La Plata (CCT CONICET-UNLP), Argentina

MC-13 Effect of formulating of Beauveria bassiana conidia on their viability and pathogenicity against the onion thrips, Thrips tabaci Reyhaneeh Ezatt-Tabin1; Reza Talei-Hassanlou1; Aziz Kharazi-Pakdel1; Khalil Taleb1; 1University of Tehran, Karaj, Iran

MC-14 Incidence, persistence and efficacy of Beauveria bassiana in cherry orchard soils Joan Cossentine1; Paul Randall1; 1Pacific 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-Aguiló1; Sonia Gómez-Vidal2; Leticia Asensio1; Pablo Barranco2; Luis V. Lopez-Llorca2; 1Universidad de Alicante, Spain; 2Universidad de Almería, Spain

MC-16 Use of Beauveria bassiana as a tool for biological control of Rhynchophorus ferrugineus Berenice Güerri-Aguiló1; Leticia Asensio1; Pablo Barranco2; Sonia Gómez-Vidal2; Luis V. Lopez-Llorca2; 1Universidad de Alicante, Spain; 2Universidad de Almería, Spain

MC-17 Pathogenicity of Beauveria bassiana and Cladosporium cladosporioides to the two-spotted spider mite Tetramychus urticae Ricardo A. Polanczyk1; Julieler G. Cocho1; Dirceu Prattisoli1; Launa P. de Souza1; Luiz Flávio V. Silveira1; Cláudio R. Franco1; Sergio B. Alves1; 1Centro de Ciências Agrárias - Universidade Federal do Espírito Santo, Brasil; 2Escola Superior de Agricultura Luís de Queiroz (ESALQ/USP), Brasil

MC-18 STU Occurrence and distribution of Beauveria and Metarhizium in Moroccan soil Imoulan Abdessalam1; Alaoui Abdelaziz1; Elmeziane Abdellatif1; 1University Cadi-Ayyad, Marrakesh, Morocco
EVALUATION OF M. ANISOPTERUS sp. AS A NATIVE SARCEVIRUS HOST

N-01 Evaluation of M. anisorhynchus as a native Sarcevirus host

N-02 Habitat complexity effects on movement of Hexameris fuscata

N-03 Pathogenicity of Thripinema fuscum

N-04 STU Susceptibility of the Colorado Potato Beetle to the nematode Pristionchus uniformis

OTHER

O-01 Toxicity of azadirachtin and some of its molecule analogue portions on larvae of Galleria mellonella (Lepidoptera) and on insect cell cultures

O-02 Cloning and expression of a venom protein from the endoparasitoid, Pimpla hypochondriae, which has haemocyte anti-aggregation activity in vitro

WEDNESDAY PM

MC-19 Evaluation of Metarhizium anisopliae for wireworm control in Switzerland

MC-20 STU Evaluating bioassay techniques for infection of Rhipicephalus ticks (Acari: Ixodidae) with entomopathogenic fungi

MC-21 Evaluation on the potential of native fungal isolates against the Mexican bean bruchid, Zeabrus subfasciatus (Coleoptera: Bruchidae) in Ethiopia

MICROSPORIDIA

M-01 Vairimorpha invictae - not detected in the parasitic fly, Pseudacteon obtusus, rearred from the microsporidium-infected fire ants, Solenopsis invicta

M-02 A new Cystosporogenes isolate from Agrius anuxius (Coleoptera: Buprestidae)

M-03 Modeling horizontal transmission of microsporidia in Lymantria dispar

NEMATODES

N-01 Hexameris sp. an entomopathogenic nematode associated with the European stink bug Simona landi

N-02 Habitat complexity effects on movement of Steiner nema carpocapsae in maize

N-03 Pathogenicity of Thripinema fuscum Tipping & Nguyen (Tylenchida: Allantonematidae) infecting Frankiniella fusca (Hinds) (Thysanoptera: Thripidae)

N-04 STU Susceptibility of the Colorado Potato Beetle to the nematode Pristionchus uniformis

OTHER

O-01 Toxicity of azadirachtin and some of its molecule analogue portions on larvae of Galleria mellonella (Lepidoptera) and on insect cell cultures

O-02 Cloning and expression of a venom protein from the endoparasitoid, Pimpla hypochondriae, which has haemocyte anti-aggregation activity in vitro

VIRUSES

V-01 Characterization of white spot syndrome virus envelope protein VP51A and its interaction with viral tegument protein VP25

V-02 Transactivation, dimerization, and DNA-binding activity of WSSV immediate early protein IE1

V-03 Characterization of the Amsacta moorei entomopox virus spheroconid promoter

V-04 Effects of chitinase J on the insecticidal efficacy of Autographa californica multiple nucleopolyhedrovirus

V-05 Reprogramming expression of chitinase and cathepsin of the Autographa californica multiple nucleopolyhedrovirus

V-06 Transactivation of Epipola apongura granulovirus (EapGV) promoters in Anticarsia gemmatalis cells

V-07 STU Early gene hhil of HzNV-1 virus is a strong apoptosis inducer and crucial for latent viral re-activation

V-08 Functional analysis of two iap genes (iap2 and iap3) of Lymantria xyline multiple nucleopolyhedrovirus

V-09 Functional analysis of the putative antipoticotic genes, p49 and iap4, of Spodoptera litura nucleopolyhedrovirus with RNAi

V-10 STU Anterograde trafficking of Autographa californica multiple nucleopolyhedrovirus is microtubule-dependent

V-11 STU Structural analysis for cypovirus polyhedrin

- 20 -
Synchrotron Radiation Research Institute, Hyogo, Japan; "Department of Agricultural Biology, NIAST, Korea; "Dong-A University, Korea

V-12 Identification of viral factors required for the enhancer-like function of baculovirus polyhedrin upstream (pu) sequence Carol P. Wu¹; Tou-Ya Huang¹; Jen-Yeu Wang¹; Hae-Ru Lo¹; Yu-Chan Chao¹; "Academia Sinica, Nankang, Taipei, Taiwan, R.O.C.

V-13 Identification of putative miRNA sequences in four insect pathogenic viruses Woonjin Kim¹; John P. Burand¹; "University of Massachusetts - Amherst, Fernald Hall, Amherst MA, USA

V-14 MicroRNAs expressed in larval gypsy moth cells post parasitization by Glyptapanteles flavicocxis parasitoid Dawn Gunderson-Rinaldi¹; "USDA, Beltsville, MD, USA

V-15 Metagenomics of glasy-winged sharpshooter, Homalodisca vitripennis (Homéptera: Cicadellidae) Wayne B. Hunter¹; Kent S. Shelby²; Scot E. Dowd¹; Catherine S. Katsar³; Pat M. Dang⁴; Laura E. Hunnicutt⁴; "USDA, ARS, Ft. Pierce, FL, USA; "USDA, ARS, Columbia, MO, USA; "USDA, ARS, Lubbock, TX, USA; "USDA, APHIS-PPQ, Fort Lauderdale, FL, USA; "USDA, ARS, Dawson, GA, USA; "North Carolina State University, Raleigh, NC, USA

V-16 Malacosoma neustria nucleopolyhedrovirus (MnNPV): Replication in Md203 cell line and host range in cell culture Rizemie Nalciaglici¹; Nurten Gurel¹; Ikbal Agah Ince¹; Ismail Demir²; Zihni Demirbag³; Karadeniz Technical University, Trabzon, Turkey; "Giresun University, Turkey

V-17 STU The characteristics and viral susceptibility of the LD cloned cells, IPLB-LD-62Y cell strains-a-f Yi-Ting Yang¹; Kuang-Hung Lin¹; Wei-Fone Huang¹; Chung-Hsiung Wang¹; "National Taiwan University, Taipei, Taiwan (R.O.C)

V-18 Applying an Anticarsia gemmatalis multiple nucleopolyhedrovirus (AgMNPV)-based direct cloning system to make a cDNA expression library of the cottonwood borer beetle (Plectrodera scutellata) Jeffrey M. Slack¹; Olga Lihoradova¹; Irina Ogay¹; Shakhrnoz Azimova¹; John Dedes¹; Rian Schwarz¹; "Great Lakes Forestry Centre, Sault Ste Marie, ON, Canada; "University of Agriculture and Technology, Fuchu, Tokyo, Japan

V-19 Optimization for high-throughput expression of recombinant protein using EasyBac system Jae Young Cho¹; Yang-Su Kim¹; Hee Jin Shim¹; Yong Wang¹; Jong Yul Roh¹; Soo Dong Woo¹; Byung Rae Jiu¹; Yeon Ho Je¹; "Seoul National University, Korea; "Seoul National University, Korea; "Chungbuk National University, Korea; "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 Liao¹; Yi-Ting Lin¹; Tsung-Yuan Wu¹; "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-Meca¹; Richard B. Hitchman¹; Linda A. King¹; "Oxford Brookes University, Oxford, UK; "Oxford Expression Technologies Ltd., Oxford Brookes University, Oxford, UK

V-22 Molecular cloning and characterization of a glycosyl hydroxylase family 9 cellulase expressed throughout the digestive tract of the emerald ash borer, Tetraopes tetrophthalmus emma Namjung Kim¹; Young-Moo Choo¹; Kwang-Sik Lee²; Seong-Jin Hong¹; Kwang-Youl Seol¹; Byung-Rae Jiu¹; "Atlantic Forestry Centre, Sault Ste. Marie, Ontario, Canada; "Atlantic Forestry Centre, New Brunswick, Canada; "University of Guelph, Ontario, Canada

V-23 Obtaining of recombinant human Müllerian Inhibiting Substance (MIS) by using baculovirus expression system Olaa A. Lihoradova¹; Irina D. Ogay¹; Maria M. Podpisnova¹; Shakhrnoz S. Azimova¹; "The Academy of Sciences of Uzbekistan, Uzbekistan; "University of Cambridge, UK

V-24 STU Persistent infection and vertical transmission of Spodoptera exigua multiple nucleopolyhedrovirus (Hübner) (Lepidoptera: Noctuidae) Oihana Cabodebilla¹; Oihane Sunim¹; Delia Muižs¹; Primitivo Caballero¹; Trevor Williams¹; "Universidad Pública de Navarra, Spain; "Instituto de Ecología AC, Veracruz, Mexico

V-25 Hypermobility and climbing behaviour induced by baculovirus infection are regulated by separate gene functions Kelli Hooyer¹; Monique M. van Oers²; "Pennsylvania State University, University Park, PA, USA; "Wageningen University, The Netherlands

V-26 Comparative pathology of the slow-killing Adoxophyes honmai NPV and Autographa californica MNPV in A. honmai Daigo Fujita¹; Takayoshi Ishii¹; Yasuhisa Kunimi¹; "Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan

V-27 Low oral infectivity of AcMNPV in Anticarsia gemmatalis larvae correlates with hemocyte resistance to infection by budded virus Eric J. Haas-Stapleton¹; Maggie Carrera¹; Tiffany Chen¹; Aniska Chikhalya¹; Alisa de la Cruz¹; Marianne Torres¹; "California State University, CA, USA

V-28 STU Investigations on the mechanism of CpGV resistance in Cydia pomonella Sabine Asser-Kaiser¹; Gary Kaene¹; Doreen Winstanley¹; Johannes A. Jehle¹; "Agricultural Service Center Patinance (DLR Rheinfalz), Neustadt an der Weinstrasse, Germany; "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. Keane¹; Sabine Asser-Kaiser¹; Marie Berling¹; Miguel Ferber Lopez¹; Johannes Jehle¹; Doreen Winstanley¹; "Warwick HRI, University of Warwick, Wellesbourne, Warwickshire, UK; "Laboratory of Biotech. Crop Protection, Dept Phytopathology, DLR Rheinfalz, Breitenberg, Germany; "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 Schmitt¹; Benoît Sauphanor¹; Johannes A. Jehle¹; Juerg Huber¹; "JKI, Institute for Biological Control, Darmstadt, Germany; "National Institute of Agronomic Research, Avignon, France; "DLR Rheinfalz, Laboratory for Biotechnological Crop Protection, Germany

V-31 Stability of resistance of codling moth against CpGV with and without virus pressure Karin Undorff-Schäli¹; Eva Fritsch¹; Juerg Huber¹; "JKI, Institute for Biological Control, Darmstadt, Germany

V-32 Comparative sequence analysis of two entomopoxviruses (EPVs) Zhen Li¹; Christopher Lucarotti¹; Peter J. Krell¹; Basil M. Arif¹; "Great Lakes Forestry Centre, Sault Ste. Marie, Ontario, Canada; "Atlantic Forestry Centre, New Brunswick, Canada; "University of Guelph, Ontario, Canada

V-33 STU A new entomopoxvirus isolated from tea tortrix, Homona coffearia, in Sri Lanka Kiri Asama¹; Keerthi Mohotti¹; Yasuhisa Kunimi¹; Madoka Nakai¹; "Tokyo University of Agriculture and Technology, Japan; "Tea Research Institute of Sri Lanka, Talawakelle, Sri Lanka
V-34 STU Comparison between two new isolates of PhopGV from Tectia solanivora and Phthorimaea operculella. Carlos Espinel-Correal1; Xavier Léry2; Laura F. Villamizar3; Alba M. Cotes4; Miguel López-Ferber4; LGEL, Ecole des Mines, Alès, France; 1IRD, Centre de Recherche, Alès, France; 2CORPOICA-CBB, Cundinamarca, Colombia

V-35 STU Determining the influence of transposon TC14.7 insertion on the function of the genome of Cydia pomonella granulovirus. Wael H. El-Mensy1,2; Johannes A. Jehle1; 1Agricultural Service Center Palatinate (DLR-Rheinpfalz), Germany; 2Agricultural 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-Alla1; François Cousserans2; Andrew G. Parker1; Alan S. Robinson1; Max Bergein1; 1FAO/IAEA Agriculture & Biotechnology Laboratory, Agency’s Laboratories, Vienna, Austria; 2Université Montpellier II, France

V-37 On the wings of Real Time: Detection, quantification, and effects of DWV. Aliva El Nagar1; Andrea Baker2; Matt Hall3; Declan Schroeder4; 1The Marine Biological Association, Citadel Hill, Plymouth, UK; 2University of Georgia, Athens, GA, USA; 3Monsanto Company, Chesterfield, MO, USA; 4Valent BioSciences, USA

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 Wednesdays, 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. Brown1, Becker Underwood, Littlehampton, UK

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

20:00 175 Field performance of novel stacked Bt products for protection against corn insects. Ken Narva1; Mike Culy1; Paul Neese1; Ed King1; Gary Thompson1; Dow AgroSciences, Indianapolis, IN, USA

20:15 176 Cancelled

20:15 177 Development of and prospects for the BtBooster platform technology. Milton D. Taylor1; Mohd Amir F. Abdullah1; Laura N. Frame1; Michael J. Adang2; 1InsectGen, Inc. Athens GA, USA; 2The University of Georgia, Athens GA, USA

20:30 178 RNAi and Bt protein approaches to corn rootworm control. Thomas L. Clark1; 1Monsanto Company, Chesterfield, MO, USA

20:45 179 Bacillus thuringiensis - based products: Forever young. Dirk Ave1, 1Valent 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. Beason1, Valient BioSciences Corporation, Long Grove IL, USA

8:30 181 Bacterial insecticides, commercial development and quality control. Changvan Chen1, Certis USA LLC, Columbia, MD, USA

9:00 182 Impact of regulations on commercialization of bacterial insecticides. Sergio Franceschini1, Intrachem Production, Grassobio, Italy

9:30 183 Proposals for a balanced regulation of microbial biocontrol agents - results of the REBECA Action. Ralf-Udo Ehlers1, University of Kiel, Germany

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

Comparative Genomics of DNA Viruses

Organizer/Moderator: Elisabeth Hemiu.

8:00 184 Evidence for extensive lateral acquisition of cellular genes by nucleocytoplasmic large DNA viruses. Jonathan Filée1; Michael Chandler1; 1LEGIS / CNRS, Gif sur Yvette, France; 1LMGM / CNRS, Toulouse, France

8:24 185 Mimivirus and Mimiviridae: Toward a new family of large DNA viruses. Jean-Michel Claverie1; Chantal Abergel1, 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. Harrison1, USDA, ARS, Beltsville, MD, USA

9:12 187 The genome of Oryctes rhinoceros: A missing link that solves some mysteries of invertebrate virus evolution. Yongjie Wang1; Monique van Oers1; Regina G. Kleespies1; M. B. Ramle2; Just M. Vlak3; Johannes A. Jehle4; 1DLR Rheinpfalz, Neustadt, Germany; 2Wageningen University, The Netherlands; 3Julius Kuehn Institute, Darmstadt, Germany; 4Malaysian Palm Oil Board, Kuala Lumpur, Malaysia

9:36 188 Wasp-bracovirus associations: The grail quest for the ancestor virus. Annie Bézier1; Marc Annaheim1; Juliene Herbinet1; Christoph Wetterwald1; Gabor Gyapay1; Sylvie Bernard-Samain1; Patrick Wincker1; Isabel Roditi2; 1IRBI CNRS, University of Tours, France; 2University of Bern, Switzerland; 3University of Bern, Switzerland; 4Genoscope, Evry, France; 5Faculté de Médecine Secteur Nord, Marseille, France; 6BIVI INRA, Université de Montpellier II, France
THURSDAY AM

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. Baker1; Aliya El Nagar1; Luke McKenzie2; Matt J. Hall3; Declan C. Schroeder4; 1Marine Biological Association of the United Kingdom, Plymouth, UK

8:15 190 STU Deformed wing virus in the parasitic mite, Tropilaelaps spp. Eva Forsgren1, Joachim R. de Miranda1,2; Mats Isaksson1; Shi Wei1; Ingemar Fries1; 1Swedish University of Agricultural Sciences, Uppsala, Sweden; 2Queen’s University, Belfast; 3National Veterinary Institute, Sweden; 4CAAS, Beijing, China

8:30 191 STU Honeybee immunity and parasitism by Nosema spp. fungi and Varroa mites Catherine M. Little1; Dave Shutler1; 1Acadia University, Wolfville, NS, Canada

8:45 192 STU Does fumagillin control the microsporidian Nosema ceranae in western honey bees (Apis mellifera)? Geoffrey R. Williams1; Michelle A. Sampson1; Dave Shutler1; Richard E.L. Rogers2; 1Acadia University, Wolfville, Nova Scotia, Canada; 2Wildwood Labs Inc.; 3Nova Scotia, Canada

9:00 193 STU Environmental effects on fungal infections in honeybee larvae (Hymenoptera: Apidae) Svjetlana Vojvodic1; Annette Bruun Jensen1; Jørgen Eilenberg1; 1University of Copenhagen, Denmark

9:15 194 Asexual reproduction in the honey bee fungal pathogen Ascosphaera apis Katherine A. Aronstein1, Keith D. Murray1,2; Robert A. Cramer1, Thomas Eubanks3; 1USDA/ARS, Welseyco, TX, USA; 2Weslaco, TX, USA; 3Tennessee State University, Bozeman, MT, USA; 4University of Texas-Pan American, Edinburg, TX, USA

10:00-10:30 BREAK BEB Lobby

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. Mevling1; Jørgen Eilenberg1; 1University of Copenhagen, Denmark

14:24 196 Covert viruses in wild populations Rosie S. Hails1; 1NERC Centre for Ecology and Hydrology, Oxford, UK

14:48 197 Microsporidian disease in beneficial insects Leellen F. Soltes1; 1Illinois Natural History Survey, Illinois, USA

THURSDAY PM

Contributed Papers Thursday, 14:00-15:45. 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 Álvaro M. Piñeiro1; Gloria M. Morales2; Sergio Ordúz3; 1Universidad de Santander, Bucaramanga, Colombia; 2Universidad Nacional de Colombia sede Medellín; 3Corporación para Investigaciones Biológicas, Medellín, Colombia

14:15 201 Characteristics of a sigf, mutant in Bacillus thuringiensis HD-73 Qi Peng1,2; Li Zhu1; Fuqing Song1; Jie Zhang1; Jiguo Gao1; Dafang Huang1; 1Chinese Academy of Agricultural Sciences, Beijing, China; 2Northeast Agricultural University, Harbin, China

14:30 202 The characteristics of an antagonistic Bacillus thuringiensis strain against crop pathogens and pests Miao M. Hang1; Liang Xiao1; Jun Cai1,2; Chi C, Xie1; Yuehua Chen3; 1Nankai University, P.R.China; 2Ministry of Education, P.R.China

14:45 203 Characterization of mosquitoicidal Bacillus cereus toxic to Ochrotatus taeniorhynchus and Culex quinquefasciatus Hyun-Woo Park1; Sabrina R. Hayes1; Florida A & M University, Panama City, FL, USA

15:00 204 Pathogenesis of male-killing Wolbachia in Drosophila bifasciata Aurore Dubuffet1; Zoe Veneti1; Henk R. Braig2; Judith E. Smith1; Greg D. D. Hurst1; 1University of Leeds, UK; 2University of Liverpool, UK; 3University 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 Ruía1; Alberto Satta1; Ignazio Floris1; David J. Ellar2; 1University of Sassari, Italy; 2University 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 Liu1; Zhaohui Wang1; S. Sivasukumar1; Liljana Georgievsk1; Glenn F. King1; W. Allen Miller2; Bryony C. Bonning2; 1Iowa State University, Ames, IA, USA; 2Institute for Molecular Bioscience, Brisbane, Australia

14:15 207 Yersinia n. sp. EN65 a novel insecticidal bacterium: A new biocontrol agent for diamondback moth, Platalea xylostella? Michael Brownbridge1,2; Sabrina R. Hayes1; 1NERC Centre for Ecology and Hydrology, Oxford, UK; 2Bio Research Limited, New Zealand

14:30 208 Biochemical characterization and insecticidal activity of an alkaline cysteine protease produced by Photorhabdus luminescens 0005-PSG isolated from Taiwan Feng-Chia Hsieh1; Yu-Tzu Chang2; Suey-Sheng Kao1; 1Taiwan Agricultural Chemicals and Toxic Substances Research Institute, Council of Agriculture, Taiwan; 2Asia University, Taiwan
14:45 **209** Heterologous expression of recombinant bacterial endochitinas and production of chitin-derived oligosaccharides J. Eleazar Barbosa-Corona1; O. B. Gutierrez-Acosta1; M. Imperial-Cervantes1; Dennis K. Bideshi1,2; N. de la Fuente-Salcido1,3; R. Salcedo-Hernandez1; 1Universidad de Guanajuato, Guanajuato, Mexico; 2Universidad Autonoma de Coahuila, Mexico; 3California Baptist University, Riverside, California, USA; 4University of California, Riverside, USA

15:00 **210** Plusine baculoviruses: Potential for cabbage looper, Trichoplusia ni, control in greenhouse vegetable production Martin A. Erlandson1; Dave Gillespie1; David Theliman1 1Agriculture and Agri-Food Canada, Saskatoon Research Centre, SK, Canada; 2Agriculture and Agri-Food Canada, Agassiz, BC, Canada; 3Agriculture 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 operculella) Steven P. Arthurs1; Lawrence A. Lacey1; 1USDA-ARS, Wapato, WA, USA

15:30 **212** Canceled

15:45 **213** Finding a microbial control agent for the invasive crayfish, Orconectes virilis Elizabeth W. Davidson1, Jennifer L. Snyder1, Donald Lightner1, Marcia Kyle1; 1Arizona State University, Tempe, AZ, USA; 2University of Arizona Agriculture Center, Maricopa, AZ, USA; 3Veterinary 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. Grant1; Justin G. Greaves1; David Chandler1; Gillian Davidson1; G Mark Tatchell1; 1University of Warwick, Coventry, UK; 2Warwick 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 Ave1; 1Valent 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 Bel1; Blair D. Siegfried1; Juan Ferre1; Baltasar Escriche1; 1University of Valencia, Spain; 2University 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-Rodriguez1; Adri Van Vliet1; Nadine Bautsens1; Jeroen Van Riel1; Juan Ferre1; 1Universitat de València, Spain; 2Bayer Bioscience N.V., Gent, Belgium

17:15 **222** The importance of antibiotic and inter-specific competition in the ecology of Bacillus thuringiensis Ben Raymond1; Michael B. Bonsall1; 1Oxford University, UK

17:50 **223** REPAT proteins and their role in the tolerance of Spodoptera exigua to its pathogens Carmen S. Hernandez1; Patricia Hernandez-Martinez1; Gloria Navarro-Cerrillo1; Juan Ferre1; Baltasar Escriche1; William J. Moor1; Ruud A. de Maagd1; Salvador Herre1; 1Universitat de Valencia, Spain; 2Auburn University, Auburn, AL, USA; 3Plant Research International B.V., Wageningen, The Netherlands

17:45 **224** Cloning and expression of the Cry1Ac-binding alkaline phosphatase (HvALP) from Heliotis virescens Omathphage P. Perera1; Jonathan D. Willis1; Michael J. Adang1; Juan Luis Jurat-Fuentes2; 1USDA-ARS Stoneville, MS, USA; 2University of Tennessee, Knoxville, TN, USA; 3University of Georgia, Athens, GA, USA

18:00 **225** Cloning of a Cry3Aa-receptor cadherin from Tenebrio molitor Jef Fbrick1; Cris Oppert1; Marcel Lorenzen1; Brenda Oppert1; Juan Luis Jurat-Fuentes2; 1USDA-ARS Maricopa, AZ; 2University of Tennessee, Knoxville, TN, USA; 3USDA-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

**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. Lietze1; Christopher C. Geden1; Drion G. Boucias1, 1University of Florida, Gainesville, FL, USA; 2USDA-ARS, Gainesville, FL, USA

16:45 **228** MdSGHV transcriptome during viral infection in the house fly Tamer Z. Salim1,2; James E. Maruniak1; Verena U. Lietze1; Drion G. Boucias1; 1University of Florida, Gainesville, Florida, USA; 2AGERI, 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 Hajiine Mor1; Hiroshi Iiri1; Gento Nishimura1; Takeshi Nakatani1; Keiko Ikeda1; Fasseli Coulibaly1; Elaine Chiu1; Peter Metcalf1; 1Kyoto Institute of Technology, Japan; 2Protein Crystal Corporation, Osaka, Japan; 3University of Auckland, New Zealand
17:30 **231** Flies infected with *Wolbachia* are less susceptible to *Drosophila C virus* Karyn N. Johnson¹; Jeremy C. Brownlie¹; Lauren M. Hedges¹, ¹University 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. Ryabov¹; Gary Keane¹; Neil Naish¹; Doreen Winstanley¹; ¹University 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

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**IMPORTANT NOTE:** Remove all posters before 18:00

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19:00 BANQUET & Britannia Royal Court Hotel

AWARDS CEREMONY

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!
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
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 thought 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 cause. 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.

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 characteristic and unique mode of infection. *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 characteristic and unique mode of infection. *Microsporidia* may infect all life forms and undoubtedly, only a small fraction of the characteristic and unique mode of infection. *Microsporidia* may infect all life forms and undoubtedly, only a small fraction of the characteristic and unique mode of infection. *Microsporidia* may infect all life forms and undoubtedly, only a small fraction of the characteristic and unique mode of infection. *Microsporidia* may infect all life forms and undoubtedly, only a small fraction of the characteristic and unique mode of infection. *Microsporidia* may infect all life forms and undoubtedly, only a small fraction of the characteristic and unique mode of infection. *Microsporidia* may infect all life forms and undoubtedly, only a small fraction of the characteristic and unique mode of infection. *Microsporidia* may infect all life forms and undoubtedly, only a small fraction of the characteristic and unique mode of infection. *Microsporidia* may infect all life forms and undoubtedly, only a small fraction of the characteristic and unique mode of infection. *Microsporidia* may infect all life forms and undoubtedly, only a small fraction of the characteristic and unique mode of infection. *Microsporidia* may infect all life forms and undoubtedly, only a small fraction of the characteristic and unique mode of infection.
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 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.

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
1 Algoma University College, Sault Ste. Marie, Ontario P6A 2G4, Canada and Department of Biological Sciences, Simon Fraser University, 8888 University Drive, Burnaby, BC, V5A 1S6, Canada
2 Departments of Zoology and Agroecology, University of British Columbia, 6270 University Boulevard, Vancouver, BC, V6T 1Z4, Canada.
Address for correspondence: jenny.cory@algomau.ca

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

Jørgen Eilenberg1; Gisbert Zimmermann2; Tariq Butt; Kerstin Jung2; Charlotte Nielsen1; Hermann Strasser1; Milton Typas3

1Department of Ecology, University of Copenhagen, Thorvaldsensvej 40, DK 1871 Frb. C., Denmark, 2BBA, Institute for Biological Control, Heinrichstrasse 243, D-64287 Darmstadt, Germany, 3Department of Biological Sciences, University of Swansea, Singleton Park, Swansea, Wales, UK SA2 8PP, UK.

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 collection culture. 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?

Intriguing interactions involving *Pandora neoaphidis* at the population scale

Jason Baverstock1; Judith K. Pell1

1 Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK.

According to the cultivated 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% 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/KK/2007/005886). The selected adsorbent showed the highest harvesting efficiency of 92.7%. The chitinase activity of the freeze-dried powder was maintained up to 80.2% 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.

Host plant effects on two isolates of *Neozeygites 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.

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

Patricia M. Wells¹; Jason Baverstock¹; Michael E.N. Majerus²; Helen E. Roy³; Judith K. Pell³

¹Rothamsted Research, Harpenden, Hertfordshire, AL5 2QJ, UK,
²University of Cambridge, Downing Street, Cambridge, CB2 3EH, UK,
³Centre for Ecology and Hydrology, Monks Wood, Huntingdon, Cambridgeshire, PE28 2LS, UK.

Address for correspondence: trish.wells@bbsrc.ac.uk

*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.

**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; Teaphritidae) adults**

Almudena Ortiz-Urquiza¹; Ana Borrego²; Cándido Santiago-Alvarez³; Enrique Quesada-Moraga¹

¹University of Córdoba, Campus de Rabanales, Building C4
²Cestelino Mutis, Second floor. 14071 Córdoba, Spain.

Address for correspondence: cr2qumes@uco.es

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 and 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 throughout 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 CSPE from media with the highest C/N values, C and D medium, in fact SDS-PAGE of 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.

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

Bryony Taylor¹; Belinda Luke¹

¹CABI, Bakeham Lane, Egham, Surrey, TW20 9TY, UK.

Address for correspondence: b.taylor@cabi.org

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 IMI386243 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.
Effects of a microsporidium from the convergent lady beetle *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae) on three non-target coccinellids

Taro Saito1, Susan Bjornson1

1Saint Mary's University, 923 Robie Street, Halifax, NS B3H3C3, Canada.

Address for correspondence: susan.bjornson@smu.ca

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.

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

Jeffrey Le1; Susan Bjornson1

1Saint Mary's University, 923 Robie Street, Halifax, NS B3H3C3, Canada.

Address for correspondence: HTJLE@DAL.CA

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. More 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.
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 Dictyocaulus, a group of microsporidia from crustacean. However, the identities of the SSU-rDNA 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.

Rapid DNA extraction from microsporidian spores of insect origin

Wei-Fone Huang1, Leelenn Solter2; Chih-Yuan Wang1; Yi-Ting Yang1; Chung-Hsiang Wang1

1Department of Entomology, National Taiwan University, No. 1, Sec 4, Roosevelt Rd., 106, Taipei, Taiwan. 2Division of Biodiversity and Ecological Entomology, Illinois Natural History Survey, 1816 S. Oak St., Champaign, Illinois, 61820, USA.

Address for correspondence: wfhuang@ntu.edu.tw

Extraction of DNA from the microsporidian spores of insect origin has required an involved process utilizing 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 107 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 SSU-rDNA. The kit requires minimal procedures and saves time for the DNA extraction of microsporidian spores, facilitating DNA analyses.

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

Yuiri S. Tokarev1; Julia M. Malyshev1; Philippe Audiot2; Igor V. Senderskii3; Andrei N. Frolov3; Sergine Ponsard1; Denis Bourque1

1All-Russian Institute for Plant Protection RAAS, Podbelskoogo 3, St. Petersburg-Pushkin 196608 Russia. 2Centre de Biologie et de Gestion des Populations, UMR INRA-IRD-CIRAD-Montpellier SupAgro, Montferrier-sur-Lez, France. 3Laboratoire Evolution & Diversité Biologique, UMR 5174, Université Paul Sabatier - Toulouse III, France.

Address for correspondence: jumaco@yahoo.com

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 rDNA 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. bombyi, and one – (82%) to Eucephalitozoon 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 paper. Monday, 15:45. 23

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

Lorena Uribe-Lorto1; Sam K. Kim2; Yolanda Flores-Lara3

1Department of Entomology, University of Arizona, 1140 E. South campus Dr. Tucson AZ 85721, USA.

Address for correspondence: spstock@ag.arizona.edu

Four Xenorhabdus spp. were extracted from newly recovered Costa Rican Steinernema species: S. websteri (CR5), S. costaricense n.sp. (CR9), S. puntavense 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. websteri symbiont in one clade, T4 symbiont in another clade alone, and S. puntavense and S. costaricense symbionts placed in a third clade, but belonging to two different clusters. S. puntavense 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.
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. entomophaga* 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 chemotraction 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 machinary.

Contributed paper. Monday, 14:45. 27

**Suppressive effects of metabolites from *Photobacterium* spp. and *Xenorhabdus* spp. on phytopathogens of peach and pecan**

David Shapiro-Flan, Charles C. Riley, and Michael W. Hotchkiss; USDA-ARS, S.E. Fruit and Tree Nut Research Laboratory, Byron, GA, 31008, USA.

Our objective was to determine the suppressive abilities of bacterial metabolites derived from *Photobacterium* and *Xenorhabdus* spp.on *Glomerella cingulata*, *Phomopsis* sp., *Phytophthora cactorum*, and *Fusarium sporum* *effusum*, which are fungal or oomycete pathogens of pecan, and *Montinillia 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 oomycete incited diseases in pecan and peach can be controlled with metabolites of *Xenorhabdus* spp. and *Photobacterium* spp.

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 amphibitic reproducing groups. The implications of this variation for the population dynamics of the hyperparasite will be discussed.

Contributed paper. Monday, 14:30. 26

**Bacillus bacteria and their fitness consequences on *Pristionchus* nematodes**

Robbie Rae1; Ralf J. Sommer1

1Max Planck Institute for Developmental Biology, 35-37 Spemannstrasse, Tuebingen 72076, Germany.

Address for correspondence: robbie.rae@tuebingen.mpg.de

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

Michael Brownbridge1 AgResearch Ltd., New Zealand.

Address for correspondence: Michael.Brownbridge@agresearch.co.nz

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**

Keith G. Davies1

1 Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK.

Address for correspondence: keith.davies@bbsrc.ac.uk
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.

Use of microbial agents in urban pest management systems

Dawn H. Gouge1; 1University of Arizona, USA.
Address for correspondence: dhgouge@ag.arizona.edu

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.

Conservation biological control strategies with entomopathogenic fungi: Potential and perspectives

Judith K. Pell1; Rothamsted Research, Harpenden, Hertfordshire, AL5 2QJ, UK.
Address for correspondence: judith.pell@bbsrc.ac.uk

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.

Entomopathogenic nematodes market diversity

Petros Ame1; e-nema, Germany.
Address for correspondence: a.peters@e-nema.de

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 Scaptotrigus vicinus and Diaprepes abbreviates 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-Granulose-Virus (CpGV) was spurring 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 Capsodis 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-enforcing process. With growing competition between companies, they are likely to expand their investment in the development of new markets.

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

Panadda Boonserm1; Min Mo1; Chanikarn Boonchoy2; Julien Lescar3

1Institute of Molecular Biology and Genetics, Mahidol University, 25/25 Phuttamonthon 4 Rd., Salaya, Phutthamonthon, Nakhon Pathom, 73170, Thailand; 2School of Biological Sciences, Nanyang Technological University, 60, Nanyang Drive, 6375512, Singapore; 3Institute of Science and Technology for Research and Development, Mahidol University, 25/25 Phuttamonthon 4 Rd., Salaya, Phutthamonthon, Nakhon Pathom, 73170, Thailand.
Address for correspondence: mbpbs@mahidol.ac.th

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 crystallography. 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 β-prism (domain II) and a plant lectin-β-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.
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 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.

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

Narumol Khomkhun1; Boonhiang Promdokny2; Chanan Angsuthanasombat1; Panadda Boonserm1
1Institute of Molecular Biology and Genetics, Mahidol University, Salaya Campus, 25/25 Putthamonthon no.4, Salaya, Nakhon Pathom 73170, Thailand, 2National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, 113 Phahonyothin Road, Pathumthani 12120, Thailand.

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

Kamomut Sinthkhamun1; Boonhiang Promdokny2
1Institute of Molecular Biology and Genetics, Mahidol University, Salaya Campus, 25/25 Putthamonthon 4, Salaya, Phuttamonthon, Nakhon Pathom 73170, Thailand, 2National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, 113 Phahonyothin Road, Pathumthani 12120, Thailand.

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 111YLD113=>111AAA113, 131NH132=>131AAA131, and 142GEQ144=>142AAA144 caused a slight reduction in toxicity compared to that of the wild type, whereas replacement at 143FQFY145=>143AAAA145 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 resulted in a total loss of toxicity. Intrinsic fluorescence 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 well 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.
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.

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

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.

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

Maria Teresa Fernandez-Luna1; Alejandra Bravo2; Humberto Lanz2; Sarjeet Gill3; Mario Soberon1; Juan Miranda-Rios1; Biotechnology Institute, National Autonomous University of Mexico, Apdo. Postal 510-3, Cuernavaca, Morelos CP 62251, Mexico. 1Instituto Nacional de Salud Publica, Av. Universidad 655,Cuernavaca, Morelos, CP 62508, Mexico. 2Department of Cell Biology and Neuroscience, University of California, Riverside, CA 92521, USA.

Address for correspondence: juanma@ibi.unam.mx

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.

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

Elisabeth A. Herniou1; Jennifer S. Cory2; Timothy G. Barracough3

1Division of Biology, Imperial College London, Silwood Park, Ascot, Berkshire, SL5 7PY, UK. 2Department of Biology, Algoma University College, Sault Ste. Marie, Ontario, P6A 2G4, Canada.

Address for correspondence: e.herniou@imperial.ac.uk

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 le-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.
Genome sequence of the complete genotype of Spodoptera frugiperda multiple nucleopolyhedrovirus isolate from Nicaragua

Oihane Simón1; Delia Muñoz2; Trevor Williams2; Primitivo Caballero1; Miguel López-Ferber1
1Instituto de Agrobiotecnología, CSIC, Universidad Pública de Navarra, Gobierno de Navarra, 31192 Mutxila Baja, Navarra, Spain, 2Instituto de Ecología AC, Xalapa, Veracruz 91070, Mexico, Ecole des Mines d'Ales, 6 avenue de Clavières, F 30319 Ales CedeX, France.
Address for correspondence: oihane.simon@unavarra.es

To understand the molecular basis for differences in speed-of-killing phenotypes the genome sequence of a fast-killing egt minus genotype of a Spodoptera frugiperda multiple nucleopolyhedrovirus from the USA (SiMNPV-3AP2) was compared with that of a slow-killing egt minus SiMNPV genotype originally isolated in Nicaragua (SiMNPV-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 SiNIC-B genome was 132,947 bp, 1,617 bp larger than that of SiMNPV-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 SiNIC-B, three of which were absent in SiMNPV-3AP2. In turn, SiNIC-B lacked the SiMNPV-3AP2 ORF129 homologue. Other genes, such as odv-e66a, p26b, were also truncated in SiMNPV-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 SiNIC-B was also observed. Construction of recombinant viruses that will help determine the genes involved in virulence is currently being undertaken.

Contributed paper. Monday, 16:45. 43

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

Karolin E. Eberle1; Doreen Winstanley2; Mohammadreza Rezapanah3; Johannes A. Jehle1
1Laboratory of Biotechnical Crop Protection, Department of Phytopathology, Agricultural Service Center Palatinate (DLR), Breitenweg 71, 67435 Neustadt/Weinstrasse, Germany, 2Warwick Horticulture Research International, University of Warwick, CV35 9EF Wellesbourne, UK, 3Insect Virology Laboratory, Biocontrol Research Department, PPDRI, Tehran, Iran.
Address for correspondence: karolin.eberle@dlr.rlp.de

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 vitro 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, 17:15. 45 STU

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

Yun-Ru Chen1; Chih-Yu Wu1; Song-Tay Lee2; Yan-Jheng Wu2; Meng-Feng Tsai1; Chu-Fang Lo1; Chung-Hsiung Wang1; National Taiwan University, No. 1, Sec. 4, Roosevelt Road, Taipei, Taiwan 10617, R.O.C.
2Southern Taiwan University of Technology, No.1, Nantai St, Yung-Kang City, Taian, Taiwan 710, R.O.C.
3Dayeh University, NO.112, Shanjiao Rd., Dacun, Changhua, Taiwan 51591, R.O.C.
Address for correspondence: wangch@ntu.edu.tw

The complete genome of the Maruca vitrata multiple nucleopolyhedrovirus (MaviMNPV) isolated from the legume pod borers, 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 BmMNPV 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, 17:00. 44

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

Yu-Shin Nai1; Tai-Chuan Wang1; Yun-Ru Chen1; Chung-Hsiung Wang1; National Taiwan University, No. 1, Sec. 4, Roosevelt Road, Taipei, 10617 Taiwan (R.O.C).
Address for correspondence: wanch@ntu.edu.tw

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 LiMNPV. The polyhedrin amino acid sequence analysis revealed that this virus belongs to Group II of baculoviruses and is closely related to LiMNPV rather than its homologous LxxyMNPV. Several other important genes of this virus had been cloned and sequenced for phylogenetic analysis. Similarly, this virus is closely related to LiMNPV as the analysis of polyhedrin. A significant deletion of fp25k sequence of this virus was found, 44 bps deletion was compared to that of LiMNPV or LxxyMNPV, 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 nucleocapsids and viruses. This virus genome size was approximately 139 kbs which is smaller than that of LiMNPV and LxxyMNPV, these results showed that this isolate is a distinct isolate but closely related to LiMNPV and named LxxyMNPV-2.

Contributed paper. Monday, 17:30. 46 STU
Orygia leucostigma nucleopolyhedrovirus (OrleSNPV) is a naturally occurring viral pathogen of the whitemarked tussock moth (Orygia leucostigma, Lymantriidae: 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 *Ectropis obliqua* (EcobNPV), *Chrysoxoides chalcites* (ChchNPV) and *Lymantria dispar* (LiMNPV).

Recently, the genome sequences of the *Glossina pallidipides* salivary gland hypertrophy virus (GpSGHV) and *Musca domestica* (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 SGHV’s 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.
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
Hinrich Schulenburg1 Westphalian Wilhelms-University, Germany. Address for correspondence: hinrich.schulenburg@uni-uebingen.de

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
Keith G. Davies1 Plant Pathology and Microbiology Department, Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK. Address for correspondence: keith.davies@bbrc.ac.uk

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 sf 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
Barton E. Slatko1, Bo Wu1, Jeremy Foster1
1New England Biolabs, Inc., Ipswich MA 01938, USA. Address for correspondence: slatko@neb.com

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
Catherine A. Eason1, David J. Clarke1
1Department of Microbiology, University College Cork, Ireland. Address for correspondence: david.clarke@ucc.ie

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 Heterorhabditidae. 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.

Invertebrate Virus Discovery

Workshop paper. Monday, 20:00. 55

Hunting for insect pathogens: A genomics approach
Wayne B. Hunter1 USDA, ARS, Fort Pierce, FL 34945, USA. Address for correspondence: wayne.hunter@ars.usda.gov

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 Picornavirus-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.
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 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 on the presence or absence of certain genes, notably p1f 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 diversification of SeMNPV from S. exigua has caused the present hypothesis that sustained diversity improvements in the 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.

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

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

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

Stephen A. Rehner; Joseph F. Bischoff; Richard A. Humber

1 USDA-ARS, Systematic Mycology and Microbiology Laboratory, Beltsville, MD 20705, USA. 2 USDA-APHIS, Beltsville, MD 20705, USA. 3 USDA-ARS, Plant, Soil Nutrition Laboratory, Ithaca, NY, 14850, USA.

Address for correspondence: Stephen.Rehner@ars.usda.gov

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.

**MICROSPORIDIA 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

Steven Valles; David Oi

1 USDA-ARS, CMAVE, 1600 SW 23rd Drive, Gainesville, FL 32608, USA.

Address for correspondence: steven.valles@ars.usda.gov

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

Yanping Chen; Jay D. Evans

1 USDA-ARS Bee Research Lab, BARC-E Bldg 476 Beltsville, MD 20705, USA.

Address for correspondence: jay.evans@ars.usda.gov

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.
Virulence Factors in Fungal Pathogens: A Comparative Approach

Symposium. Tuesday, 8:00. **60**
Pathogenicity determinants of the entomopathogenic fungi *Metarhizium anisopliae*
Raymond J. St. Leger; Chengshu Wang; Weiguo Fang; Sibhao Wang

1Department of Entomology, University of Maryland, USA.
2Shanghai Institutes for Biological Sciences, Institute of Plant Physiology and Ecology, PRC.

Address for correspondence: stleger@umd.edu

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 periplasm 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 have reached the haemocoel. Exploiting microarray and gene disruption has therefore been successful in identifying key aspects of pathogenicity.

Symposium. Tuesday, 8:24. **61**
Attenuation of virulence in entomogenous fungi
Tariq M. Butt; Farooq A. Shah

1Swansea University, SOTEAS, Singleton Park, Swansea, SA2 8PP, UK.

Address for correspondence: t.butt@swansea.ac.uk

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
Michael J. Bidochka

1Brock University, Canada.

Address for correspondence: bidochka@brocku.ca

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; Michael J. Kershaw; Diane O. Saunders; Elise Lambeth; Ana-lilia Martinez-Rocha; Nicholas J. Talbot

1Stocker Road, University of Exeter, UK.
2Departamento de Genetica, Universidad de Córdoba, Campus de Rabanales, Edificio Gregor Mendel, 14071 Córdoba, Spain.

Address for correspondence: m.j.egan@ex.ac.uk

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 *MgsATG8* 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?
Alice C.L. Churchill

1Cornell University, Department of Plant Pathology and Plant-Microbe Biology, Ithaca, NY 14853 USA.

Address for correspondence: acc7@cornell.edu

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 systems.
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.

**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 Jaoua1; Souad Rouis1; Slim Tounsi1; Nabil Zouari1; Imène Saadaoui1; Hichem Azouz2; Lobna Abdelkafi Mesrat1

1Laboratory of Biopesticides, Centre of Biotechnology of Sfax.

Address for correspondence: samir.jaoua@cb.rmutn.tn

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, cry1la, 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 grain- 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.

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

Nora C. Lawo1; Rod J. Mahon2; Richard J. Milner3; Bidyut K. Sarmah1; Thomas J.V. Higgins1; Jörg Romeis1

1Agroscope Reckenholz-Tänikon Research Station ART, Reckenholzstr. 191, 8046 Zurich, Switzerland, 2CSIRO Entomology, GPO BOX 1700, Canberra, ACT 2601, Australia, 3Department of Agricultural Biotechnology, Assam Agricultural University, Jorhat 785 013, India, 4CSIRO Plant Industry, GPO Box 1600, Canberra ACT 2601, Australia.

Address for correspondence: nora.lawo@art.admin.ch

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 *Cry2A*-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.
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.

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-contaminated 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.

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 thuringiensis (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.

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, silk gland, midgut, malpighian 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.
Unravelling interspecific variability in virulence of four entomopathogenic nematodes to four white grub species:

Virulence, infectivity, penetration sites

Albrecht M. Koppenhöfer1,2; Eugene M. Fuzy1
1Dept. Entomology, Rutgers University, Blake Hall, 93 Lipman Dr, New Brunswick, NJ 08901, USA.
Address for correspondence: koppenhoefer@aesop.rutgers.edu

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.

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, 93 Lipman Drive, Blake Hall, New Brunswick, NJ 08904, USA.
Address for correspondence: bmcgraw@eden.rutgers.edu

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.

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

Jenna Ross1; Sergei Spiridonov2; Elena Ivanova2; Jeremy Pearce2; Paul Severns3; Graeme Nicoll1; Michael Wilson1
1Institute of Biological and Environmental Sciences, University of Aberdeen, St Machar Drive, Aberdeen, UK; 2Institute of Parasitology, Russian Academy of Sciences, Moscow, Russia; 3Becker Underwood, Littlehampton, West Sussex, UK.
Address for correspondence: jenna.ross@abdn.ac.uk

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 Angiostrongyla, Paramphorhabditis and Agfa spp., whereas in the USA the majority of nematodes were found to be Allionema, Angiostrongyla 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.
Potential for biocontrol of *Diaprepes abbreviatus* larvae in nurseries in southern California

Kenneth O. Spence¹; Edwin E. Lewis¹; Jim Bethke²

¹University of California-Davis, Department of Nematology, Davis CA 95616, USA, ²UCCE-San Diego County, Suite 4101 Building 4, San Marcos CA 92123, USA.

Address for correspondence: kospence@ucdavis.edu

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.

**Corresponding author:** Kenneth O. Spence; Kenneth.spence@ucdavis.edu

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

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

Jingjiao Song¹; Ranran Wang¹; Fei Deng¹; Hualin Wang¹; Zhihong Hu²

¹Wuhan Institute of Virology, Chinese Academy of Sciences, Xiao Hong Shan District #44, Wuhan, 430071, P. R. China.

Address for correspondence: huhz@wh.iov.cn

In this manuscript, the *per os* infectivity factors (PIFs) of *Helicoverpa armigera* single nucleopolyhedrovirus (HearNPV) were studied together. HearNPV bacmids with deletions of *p74* (Ha132), *pif1* (Ha111), *pif2* (Ha132) and *pif3* (Ha98) were constructed individually by homologous recombination in *E. coli* cells. Repaired bacmids with respective *pifs* 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 *pifs* did not have any obvious affects to the morphology of the occlusion bodies. Bioassay analyses indicated that deletion of any of the above *pifs* 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.
The transmembrane domain of the AcMNPV GP64 protein plays specific roles in membrane fusion and virion budding

Zhaofei Li1; Gary W. Blissard1

1Boycie Thompson Institute at Cornell University, Tower Road, Ithaca, NY 14853, USA.

Address for correspondence: gwb1@cornell.edu

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.
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, Ying Tan, Feifei Yin, Fei Deng, Zhihong Hu, Just M. Vlaak, Hualin Wang

1 Wuhan Institute of Virology, Chinese Academy of Sciences, Xiao Hong Shan District #44, Wuhan 430071, China, 2 Laboratory of Virology, Wageningen University, 6709 PD Wageningen, The Netherlands.

Address for correspondence: h.wang@wh.iov.cn

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 vAcP48-KO was unable to propagate in cell culture, while a 'repair' Bacmid vAcP48-GFP was able to replicate in a manner similar to a wild-type Bacmid vAcP48-GFP. Titration assay and Western blot analysis confirmed that vAcP48-KO-P34-GFP was unable to produce budded viruses (BV). 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, 9:30. B4 STU

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

Meijin Yuan, Wenbi Wu, Chao Liu, Yanjie Wang, Chaoyang Hu, Kai Yang, Yi Pang

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

Address for correspondence: lissymj@mail.sysu.edu.cn

The F-like protein of group I NPVs, but to be irrelevant for budding here was a -ing effect on the c PH varroa and bees. Besides, there is re that analysis virus (IAPV, refs 1, 2) has been found to be ri, E. Tanne, I. 83x186 production and ODV envelopment in the AcMNPV life cycle. showed that P48 prominently condensed in viruses (ODVs) and their subsequent occlusion. Confocal analysis required for nucleocapsid envelopment to form occlusion DNA replication. Electron Microscopy indicated that P48 was confirmed that vAcP48-KO-P34-GFP was unable to produce budded viruses (BV). 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. B5 STU

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

David C. J. Carpentier, Caroline M. Griffiths, Linda A. King

1 Oxford Brookes University, Headington Campus, Oxford, OX3 0BP, UK.

Address for correspondence: dcarpenter@brookes.ac.uk

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 cisternal-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 bundles’. 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 PF34 (AcORF-131, PEP) with the polyhedral envelope. Early indications are that deletion of p10 increases PF34 association with polyhedra.

Contributed paper. Tuesday, 9:15. B3 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, Ying Tan, Feifei Yin, Fei Deng, Zhihong Hu, Just M. Vlaak, Hualin Wang

1 Wuhan Institute of Virology, Chinese Academy of Sciences, Xiao Hong Shan District #44, Wuhan 430071, China, 2 Laboratory of Virology, Wageningen University, 6709 PD Wageningen, The Netherlands.

Address for correspondence: h.wang@wh.iov.cn


Symposium. Tuesday, 11:00. 87

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

Laurent Gauthier, Julie Fievet, Diana Tentcheva, Marc Edouard Colin, Max Bergoin

1 SupAgro Montpellier, Laboratoire de Pathovigilance et de Développement Apicole, 900 rue Jean François Breton, 34090 Montpellier, France, 2 Université Montpellier 2, UMR BIVI INRA-UM2, CC101, Place Eugène Bataillon, 34095 Montpellier Cedex5, France.

Address for correspondence: bergoinm@supagro.inra.fr

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
Sebastian Gisder1; Constanze Yue1; Elke Genersch1
1Institute for Bee Research, Friedrich-Engels-Str. 32, 16540 Hohen Neuendorf, Germany.
Address for correspondence: elke.genersch@rz.hu-berlin.de

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
Rajwinder Singh1, Abby Kalkstein1, Edwin Rajotte1, Dennis vanEngelsdorp1, Nancy Ostiguy1, Eddie Holmes1, Claude dePamphilis2, Rick Donvall1, Ian Lipkin1, Diana Cox-Foster1
1Dept of Entomology, Penn State University; 2Dept of Biology, Penn State University, University Park, PA, USA; 3Center for Infection and Immunity, Mailman School of Public Health, Columbia University, New York, NY, USA
Address for correspondence: dxc12@psu.edu

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.

POSTERS – 1

BACTERIA

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

Generation of a Manduca sexta larval midgut EST collection
Yannick Pauchet1; Helko Vogel2; Paul Wilkinson3; David G. Heckel4; Richard H. French-Constant5
1School of Biosciences, University of Exeter in Cornwall, Penryn, TR10 9EZ, UK; 2Max Planck Institute for Chemical Ecology, Jena, D-07745, Germany.
Address for correspondence: Y.Pauchet@exeter.ac.uk

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
Kara S. Giddings1; Andrew M. Wollacott1
1Monsanto Company, 700 Chesterfield Pkwy West, Chesterfield MO 63017, USA; 2Monsanto Company, 325 Vassar St., Cambridge MA 02139, USA.
Address for correspondence: kara.s.giddings@monsanto.com

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.
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 genonic 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 as to determine the genetic linkage of the cadherin to Bt resistance traits.

Some mutations in the mosquitoicidal 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 K118A or K198A.

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.

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

Yolanda Bel1; Juan Ferré2; Baltasar Escrich1
1Genetics Department, University of Valencia, Dr. Moliner, 50 46100-Burjassot, Spain.
Address for correspondence: Yolanda.Bel@uv.es

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

Omaathhage P. Perera1; Anais S. Castagnola2; Juan Luis Jurat-Fuentes2; Craig A. Abel1
1Southern Insect Management Research Unit, USDA-ARS, 141 Experiment Station Road, Stoneville, MS 38776, USA, 2Department of Entomology and Plant Pathology, University of Tennessee, 2431 Joe Johnson Drive, Knoxville, TN 37996, USA.
Address for correspondence: op.perera@ars.usda.gov

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.

**Interaction between REPAT members, a family of pathogen induced proteins**

Gloria Navarro-Cerrillo1; Juan Ferré2; Ruud A. de Maagd2; Salvador Herrera1
1University of Valencia, Dr. Moliner 50, 46100 Burjassot, Valencia, Spain, 2Plant Research International B.V., Wageningen University, Wageningen, The Netherlands.
Address for correspondence: gloria.navarro-cerrillo@uv.es

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

Xiaochuan Li1; Dexuan Xie1; Peter Butko2
1Boston University, Boston, MA 02118, USA, 2University of Wisconsin, Milwaukee, WI 53201, USA, 3University of Maryland, Baltimore, MD 21201, USA.
Address for correspondence: pbutko@rx.umaryland.edu

In order to confirm all the possible interactions between the different members of the REPAT family.
Spodoptera exigua gene expression profile in response to sublethal intoxication by a commercial Bacillus thuringiensis based product

Patricia Hernandez-Martinez1; Gloria Navarro-Cerrillo1; Ruud A. de Maagd2; Baltasar Escriche3; Salvador Herrero1
Address for correspondence: patricia.hernandez@uv.es

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.

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

Andrea J. Dowling1; Isabella Visidu2; Nicholas R. Waterfield2; Richard H. ffrench-Constant1; William Wood2
1University of Exeter in Cornwall, School of Biosciences, University of Exeter in Cornwall, Falmouth, TR10 9EZ, UK, 2University of Bath, Department of Biology and Biochemistry, University of Bath, Bath, BA2 7AY, UK.
Address for correspondence: a.j.dowling@exeter.ac.uk

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 (Photobahabus) 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.

Study on Bt susceptibility and resistance mechanisms in the sugarcane borer, Diatraea saccharalis

Yu Cheng Zhu1; Xiaoyi Wu2; Yunlong Yang2; James Otte2; Roger Leonard1; Craig A. Abel1; Fangneng Huang2
1USDA-ARS, 141 Experiment Station Road, Stoneville, MS 38776, USA, 2Louisiana State University, Baton Rouge, LA 70803, USA.
Address for correspondence: yc.zhu@ars.usda.gov

Rapid adoption of Bt corn applied heavy selection pressure on corn borers, especially the emerging sugarcane borer (Diatraea saccharalis) in Louisiana. By using novel F2 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.

Characterization of the Heliothis virescens midgut regenerative response upon treatment with Bacillus thuringiensis Cry1Ac toxin

Anaïs S. Castagnol1; Omaththage P. Perera2; Juan Luis Jurat-Fuentes1
1Department of Entomology and Plant Pathology, University of Tennessee, 2431 Joe Johnson Drive, Knoxville, TN 37996, USA,
2USDA-ARS Southern Insect Management Research Unit, Stoneville, MS 38776, USA.
Address for correspondence: jurat@utk.edu

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.

High temperature could trigger rapid development of resistance to Bt toxin Cry1Ac and deltamethrin in Plutella xylostella

Ali H. Sayyed1; Neil Crickmore1
1University of Sussex, Falmer, Brighton, BN1 9QG, UK.
Address for correspondence: h.a.sayyed@sussex.ac.uk

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 20°C and 28°C 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 28°C than at 20°C. In contrast resistance to chlorpyrifos developed more rapidly at 20°C.

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

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

Paul R. Johnston; Vidisha Krishnan; Ruchin Mishra; Ali H. Sayed; Neil Critchmore

Biochemistry Department, University of Sussex, Brighton, BN1 9QG, UK.

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.

**Characterization of the Cry41Aa parasporin**

Vidisha Krishnan; Stella Stamatopoulou; Hideki Katayama; Eiichi Mizuki; Neil Critchmore

University of Sussex, Brighton, BN199GQ, UK; Biotechnology and Food Research Institute, Fukuoka 839-0861 Japan.

Owing to the reported cytotoxic 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 & Cry41Aa1, 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 is 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 cytoidal 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 Cry5Aa, 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 investigated the effect of removing the HA-33 like domain.

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

**The efficacy of non-mosquitoicidal 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; Nadasajh V. Devi; Mohamed S. Mariam; Abdullah Maha

International Medical University, 126 Jalan 19/155B, Bukit Jalil, 57000 Kuala Lumpur, Malaysia, Universiti Putra Malaysia, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

Address for correspondence: kokkeong_chan@imu.edu.my

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-mosquitoicidal 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 characterisation of the parasporal proteins of Bt18 showed changes in inhibition selectivity, causing an increase in percentage cell viability for CEM-SS and 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 Parasporin 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-15 STU

**Characterisation of the Cry41Aa parasporin**

Vidisha Krishnan; Stella Stamatopoulou; Hideki Katayama; Eiichi Mizuki; Neil Critchmore

University of Sussex, Brighton, BN199GQ, UK; Biotechnology and Food Research Institute, Fukuoka 839-0861 Japan.

Address for correspondence: vidisha.krishnan@gmail.com

A Malaysian *Bacillus thuringiensis* isolate designated Bt18 expresses parasporal proteins specifically cytotoxic against CEM-SS, a leukemic T lymphoblastoid cell (CD3e=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 leukemic 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 leukemic T cell lysate. N-terminal amino acid sequencing of the 34-kDa protein was GKYK/VG/NG/RIG 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.
Different mechanisms of action of Bacillus thuringiensis Cry1Ac toxin along the midgut of lepidopteran larvae
Silvia Cacciari\(^1\); Ana Rodrigo-Simón\(^1\); Juan Ferre\(^1\)
\(^1\)Universitat de València, Dr. Moliner 50, 46100 Burjassot (Valencia), Spain.
Address for correspondence: juan.ferre@uv.es

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.

Study of two midgut aminopeptidases from Ostrinia nubilalis Hübnner
Cristina M. Crava\(^1\); Yolanda Bel\(^2\); Barbara Manachini\(^2\);
Baltasar Escrichte\(^1\)
\(^1\)University of Valencia, Dr. Moliner 50, 46100 Burjassot, Valencia, Spain,
\(^2\)University of Palermo, via Archirafi 18, 90123 Palermo, Italy.
Address for correspondence: m.cristina.crava@uv.es

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 HEXXH\(_{\alpha}E\) 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.

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\(^1\)
\(^1\)Department of Entomology, Iowa State University, 418 Science II, Ames IA 50011, USA.
Address for correspondence: hrl@iastate.edu

Hemipteran pests are not susceptible to the effects of known Bt toxins and have replaced the Lepidoptera as primary pests on Bt transgenics. 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 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.

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 184-8588, Japan.
Address for correspondence: 50007401304@st.tuat.ac.jp

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 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.

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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; Ana F. Parente; Mariana T R Lira; Tayana Kariya; Isabela M M de Oliveira; Marlene T. De-Souza

Brazilia University, Cell Biology Dept., Campus Universitário Darcy Ribeiro, 70990-900 - Brasilia, DF, Brazil.

Address for correspondence: marths@unb.br

Bacillus thuringiensis as bioinsecticide 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 plasmid/s/20 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 about after 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. thuringiensis is a useful tool to study the biology of this bacterium.

Construction of modified Bacillus thuringiensis cry1Ac genes based on cry1-5 genes through multi site-directed mutagenesis

Hong Guang Xu; Jong Yul Roh; Jae Young Choi; Hee Jin Shim; Yong Wang; Qin Liu; Soo Dong Woo; Byung Rae Jin; Yeon Ho Je.

Department of Agricultural Biotechnology, Seoul National University, Seoul 151-742, Korea. Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 151-742, Korea. College of Agriculture, Life and Environment Sciences, Chungbuk National University, Cheongju 361-763, Korea. College of Natural Resources and Life Science, Dong-a University, Busan 604-714, Korea.

Address for correspondence: bxus@snu.ac.kr

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.

Construction of a Bacillus thuringiensis engineered strain with high toxicity and broad insecticidal spectrum to Coleopteran by homologous recombination

Jingjing Liu; Jie Zhang; Changlong Shu; Fuping Song; Guixin Yan; Dafang Huang

College of Life Science, Northeast Agricultural University, Harbin, 150030, China. State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, 100193, China. Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing, 100081, China.

Address for correspondence: dfhuang@ippcaas.cn

The larvae of Cockchafer are important insect pests in agriculture, horticulture, and forestry in both Europe and Asia. In China, Anomala carpentula 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 carpentula 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 pS6EC carrying cry8Ca2 gene with high toxicity to Anomala carpentula was constructed, and transformed into B. thuringiensis isolate 185. The cry8Ca2 gene was integrated into the internal of transposons Tnp157B 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 carpentula.

Engineered Bacillus thuringiensis 3A-HBF with insecticidal activity against Scarabaeidaeae and Chrysomelidae

Guixin Yan; Changlong Shu; Fuping Song; Jingjing Liu; Dafang Huang; Jie Zhang

State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China. College of Life Science, Northeast Agricultural University, Harbin 150030, China. Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing 100081, China.

Address for correspondence: jzhang@ippcaas.cn

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 carpentula. 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 carpentula. The corrected mortality to CPB larvae was 100% after 24 hours, and to A. carpentula 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.

Studies on protease-resistant core form of Bacillus thuringiensis Cry1Ie toxin

Shuyuan Gao1; Yancai Zhang3; Je Zhang3; Fuping Song2; Dafang Huang1
1School of Life Science & Technology, Beijing Institute of Technology, No.5 zhongguancun Southroad, Beijing 100081, China, 2State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, CAAS, No. 2 Yuanmingyuan Westroad, 100094, Beijing, China, 3Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Zhongguancun Southroad, Beijing, 100081, China.
Address for correspondence: guoshuyuan@tsinghua.org.cn
cry1Ie genes were silent in Bacillus thuringiensis 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 reconvered 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 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.

Evidence of the involvement of the C-terminal portion of Bacillus thuringiensis Cry1Ac delta-endotoxin in crystallization

Slim Toussi1, Mariam Damak1, Samir Jaoua1
1Centre of Biotechnology of Sfax, P.O. Box "1177", 3038, Sfax,Tunisia.
Address for correspondence: slim.toussi@cisb.mrt.tn

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 Cry1AcB 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 delta-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.

Bacillus thuringiensis serovar thompsoni HD542 crystal proteins: Solubilization, activation, and insecticidal activity

Samir Naimov1; Rumyana Boncheva1; Ruud deMaagd1
1University of Plovdiv " Paisii Hilendarski", 24 "Tzar Assen" Str, Plovdiv 4000, Bulgaria, 3Busines Unit Bioscience, Plant Research International B.V, Wageningen University and Research Centre, Wageningen 6700AA, P.O. Box 16,The Netherlands.
Address for correspondence: samir.naimov@gmail.com

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.

20kb DNA: What is it doing in Bt crystals?

Seher Fazal1; Christopher Jones1; Neil Crickmore1
1University of Sussex, Brighton, BN1 9QG, UK.
Address for correspondence: sf94@sussex.ac.uk

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.
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.

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**Characterization of environmental isolates of Bacillus thuringiensis from northeastern Poland harbouring vip3A gene homologues**

Izabela Swiecicka1; Dennis K. Bideshi2; Magdalena Czajkowska1; Sylwia Kotowicz1

1Department of Microbiology, University of Bialystok, Swierkowa 20B, PL15-950 Bialystok, Poland, 2Department of National and Mathematical Science, California Baptist University, 8432 Magnolia Ave, Riverside, California 92504, USA.

[Address for correspondence: izabelas@uwb.edu.pl]

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**Identification and cloning of novel cry genes from Bacillus thuringiensis strain Y41**

Changlong Shu1; Xiaodong Su2; Jie Zhang3; Dafang Huang4; Fuping Song5

1Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, 100193, P. R. China, 2Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing, 100081, P. R. China.

[Address for correspondence: jzhang@ippcaas.cn, fpsong@ippcaas.cn]

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 cry40Aa1, cry30Aa1, cry52Aa1 and cry53Aa1 respectively.

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**Identification of new cry genes of Bacillus thuringiensis through the use of a system of universal primers**

Pedro A. Nogueira1; Jorge E. Ibarra2

1Departamento de Biotecnologia y Bioquímica, CINVESTAV-IPN, Apartado postal 629, Irapuato, GTO. Mexico.

[Address for correspondence: pnuogueira@ira.cinvestav.mx]

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 (LB1T-series) collection log were further selected by phase contrast 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
Corina M. Berog1; Macarena Párez-Cenci1; Graciela L. Salerno1
1Fundación para Investigaciones Biológicas Aplicadas (FIBA), Viyetes 3103 (7600) Mar del Plata, Argentina.
Address for correspondence: cberon@fib.org.ar

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 OL1GC 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
Ben Ryall1; Hannah Nasser1; Dimitris Mossialos2; Huw D. Williams2
1Division of Biology, Imperial College London, Sir Alexander Fleming Building, London SW7 2AZ, UK.
2Department of Biochemistry & Biotechnology, University of Thessaly, Ploutonos 26 & Aioliou, Larissa, GR-41221, Greece.
Address for correspondence: mosial@bio.uth.gr

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 acts 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
Ana M. Guidelli-Thuler1; Janete A. Desidério Sena1; Irlan L. de Abreu1; Camila C. Davolos1; Sergio B. Alves1; Ricardo A. Polanczyk1; Fernando H. Valicente1; Manoel Victor F. Lemos3
1Universidade Estadual Julio Mesquita Filho (UNESP Jaboatobal), Vila de Acesso Prof. Paulo Donato Castellane S/N 14884-900 Jaboatobal -SP, Brasil, 2Escola Superior de Agricultura Luiz de Queiroz (ESALQ-USP), Av. Pádua Dias, 11 CP 9, 13418-900 Piracicaba-SP, Brasil, 3Centro de Ciencias Agrarias - Universidade Federal do Espírito Santo. Alto Universitário S/N 29500-000 Alegres ES, Brasil, 4Empresa Brasileira de Pesquisa Agropecuária, Embrapa Milho e Sorgo, CP 151, 35701-970 Sete Lagoas-MG, Brasil.
Address for correspondence: rapolanc@yahoo.com.br

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 cry1 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 cry1Aa, cry1Ab, cry1Ac, cry1Ad, cry1Ae, cry1Af, cry1Ag, cry1Bf, cry1Ca and cry1F 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 cry1Ab (42.12%) and lowest percentage for the cry1Db subclass (0.6%). The genetic variability of the analyzed bacterial collections seems to point that the Piracicaba and the Jaboatobal 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 cry1B 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
Marise T. Suzuki1; C. Sara Hernández-Rodríguez1; Welington L. de Araújo2; Juan Ferre3
1Universitat de València, Dr. Moliner 50, 46100-Burjassot (Valencia), Spain, 2Universidade de São Paulo, Escola Superior de Agricultura “Luiz de Queiroz”, Av. Paduas Dias, 11, 13418-900- Piracicaba/SP, Brazil.
Address for correspondence: juan.ferre@uv.es

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 stored 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 (CTH31RIB4). 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.
Electron-microscopic and genetic characterization of *Rickettsiella tipulae*, an intracellular bacterial pathogen of the crane fly, *Tipula paludosa*

Regina G. Kleespießen; Andreas Leclerque

1Federal Research Centre for Cultivated Plants, Julius Kuehn-Institute, Institute for Biological Control, Heinrichstr. 243, D-64287 Darmstadt, Germany.

Address for correspondence: regina.kleespießen@jki.bund.de

*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 isospod-associated further *R. popilliae*-synonymized pathotype, *Rickettsiella armadillidi*, suggests that the latter might better be assigned to a different species.

Posterm / Bacteria. Tuesday, 10:30. *B-38*

Functional analysis of nematocidal protein Cry6Aa2 from *Bacillus thuringiensis*

Jun Cai; Xue-Zhao Liu; Yong-Qiang Jia; Bing Yan; Yue-Hua Chen; Yu Yuan

Key Laboratory of Molecular Microbiology and Technology, Ministry of Education, College of Life Sciences, Nankai University, Tianjin 300071, China.

Address for correspondence: caijun@nankai.edu.cn

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 LC50 was 38.35ng/cm2. Sequence analysis results indicates there exist proteolytic cleavage sites at aa11 and aa382 of Cry6Aa2, which 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 toxicity of mutants R117 &L382L, which lost N-terminal or C-terminal proteolytic cleavage site respectively, were reduced. The toxicity of double mutant R117 &L382L, which lost both N-terminal and C-terminal proteolytic cleavage site, was most significant lower than that of *Cry6Aa2*, R117T 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 LC50 was 21.45ng/cm2, 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. The breaking apart of disulfide bonds and N-terminal and C-terminal activation of *Cry6A* is essential in these two steps, respectively.

Poster / Bacteria. Tuesday, 10:30. *B-39*

Characterization of two *Bacillus thuringiensis* subsp. *morrisonii* strains isolated from *Thaumetopoea pityocampa* Den. and Schiff., (Lep., *Thaumetopoeidae*)

Hatice Kati; Ikbol A. Ince; Kazim Sezen; Serifel Icgi; Zihni Demirbag

1Giresun University, Faculty of Arts and Sciences, Department of Biology, 28049, Turkey. 2Karadeniz Technical University, Faculty of Arts and Sciences, Department of biology 61080, Turkey.

Address for correspondence: ikati@ktu.edu.tr

*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. *morrisonii* (Hk88b). 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 *Aeglastica abtii* and *Lepinotarsa decemlineata* and 100% for Tp14 isolate on the larvae of *Malacosoma neustria*, respectively. Our results indicate that *B. thuringiensis* subsp. *morrisonii* strains, Tp6 and Tp14 isolates, may be valuable as biological control agent for coleopteran and lepidopteran pests.

Poster / Bacteria. Tuesday, 10:30. *B-40*

Characterization of *Bacillus thuringiensis* strain collections from Spain and evaluation of their insecticidal activity against *Ceratitis capitata*

José Cristian Vidal-Quist; Pedro Castañera; Joel González-Cabrera

1Instituto Valenciano de Investigaciones Agrarias, Ctra. Moncada-Náquera km 4,5, Valencia, Spain. 2Centro de Investigaciones Biológicas, c/ Ramiro de Maestu 9, Madrid, Spain.

Address for correspondence: joel.gonzalez@ivia.es

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 diptersan. However, no active strain against *Ceratitis capitata* Wiedemann has been described to date. In this study, a collection of 370 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.
Susceptibility to Bacillus thuringiensis of neonates and older larvae of Tortrix viridana L. (Lepidoptera: Tortricidae) from a natural reserve

Barbara Manachini1; Filippo Castiglia2
1Department of Animal Biology, University of Palermo, 18, via Archirafi, 90123, Palermo, Italy, 2Azienda Regionale Foresti Demaniiali, Ufficio Provinciale Palermo, 23, via del Duca - 90143.
Palermo, Italy.
Address for correspondence: b.manachini@unipa.it

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 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. viridana larvae were recorded. For neonates larvae the calculated DL50 was 0.63 mg/ml while the same doses had little effect on the older, 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.

New strategy for isolating novel nematicidal crystal protein genes from Bacillus thuringiensis strain YBT-1518
Suxia Guo1; Donghai Peng1; Weiya Li1; Sisi Ji1; Pengxia Wang1; Ziniu Yu1; Ming Sun1
1College of Life Science and Technology, Huazhong Agricultural University, Wuhan 430070, Hubei, P.R. China.
Address for correspondence: m98sun@mail.hzau.edu.cn

This work describes a novel strategy for cloning cry genes from Bacillus thuringiensis by constructing library with B. thuringiensis as host and shuttle vector pH7304 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, 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 bipyramidal 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.

Physiological characterization of accumulated poly-β-hydroxybutyrate(PHB) in Bacillus thuringiensis
Chen Deju1; Yan Jin1; Meng Ying1; Chen Shouwen1; Sun Ming1; Yu Ziniu1
1State Key Laboratory of Agricultural Microbiology, College of Life Science and Technology, Huazhong Agricultural University, Wuhan 430070, P. R. China.
Address for correspondence: yz41@mail.hzau.edu.cn

Poly-β-hydroxybutyrate(2HBs) are accumulated during exponential growth and then utilized very fast during the early stationary phase in Bacillus 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 knockedout gene phaC, the key gene of accumulation of PHB, by inserted an erythromycin-resistance gene to replace gene phaC 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+/NADPH 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.

The red palm weevil (RPW), Rynchophorus ferrugineus (Oliv.) (Coleoptera, Curculionidae) is one of the most serious pest for palm in urban areas. In Italy was accidentally introduced in 2004 and in less than 3 years has become a tremendous problems for ornamental palms belonging to Carica papaya. Since chemical applications are difficult in urban areas, biological control methods should be preferred. A commercial preparation of the microbial entomopathogen, Bacillus thuringiensis var. kurstaki, was evaluated for biological activity against the RPW. Based on oral bioassay was carried out with fermented mutant strain was lower than that in the parent strain. When we inserted an erythromycin-resistance gene to replace gene phaC 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+/NADPH 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.

TUESDAY AM
TUESDAY AM
**Influence of different strategies of European corn borer (Ostrinia nubilalis Hübnner) control on the content of contaminants in maize.**

Vladan Faltă; Jitka Stará; František Kocourek; Ludmila Slezáková; Jana Hajtlová; Vladimír Kocourek; O. Lacina; J. Honziček; Jana Tichá; Alexandra Krlíčková; Monika Gocieková.

Crop Research Institute, Dmovská 507, Prague, 161 06, Czech Republic; Institute of Chemical Technology Prague, Technická 65, Prague 166 28, Czech Republic.

Poster / Bacteria. Tuesday, 10:30. **B-46**

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 (methoxyfenozide, indoxacarb) used in ECB chemical control of sweet maize was analysed. In Bt maize the lower occurrence of toxicogenic 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 below 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.

**Efficacy of different strategies of European corn borer (Ostrinia nubilalis Hübnner) control in maize.**

Jitka Stará; Vladan Faltá; František Kocourek; Ludmila Slezáková.

Crop Research Institute, Dmovská 507, Prague, 161 06, Czech Republic.

Address for correspondence: stara@vurv.cz

Poster / Bacteria. Tuesday, 10:30. **B-47**

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 toxicogenic micromycetes dangerous both for human consumers and animals. The efficacy of selective insecticides (methoxyfenozide, 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.

**Identification of commercial BT-strains by molecular markers.**

Gian Paolo Barzanti1; Elena Così; Pietro Rumine2; Pio F. Roversi1

C.R.A. - Centro di Ricerca per l’Agrobiologia e la Pedologia, via Lanciola 12/A, Cascine del Riccio, 50125 Firenze, Italy.

Address for correspondence: gianpaolo.barzanti@iszra.it

Poster / Bacteria. Tuesday, 10:30. **B-48**

*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 Brouseau 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.

**Host plant preference of spider mites on Bt-expressing and control potatoes.**

Rostislav Zemek1; Institute of Entomology, Biology Centre AS CR, Braníovska 31, Ceske Budejovice, Czech Republic.

Address for correspondence: rosta@entu.cas.cz

Poster / Bacteria. Tuesday, 10:30. **B-49**

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.

**Interactions between Cry1Ac, Cry2Ab, and Cry1Fa *Bacillus thuringiensis* toxins in the cotton pests Helicoverpa armigera (Hübner) and Earias insulana (Boisdruaval).**

Maria A. Ibarutz1; Delia Muñoz1; Ilguo Ruiz de Escudero1; Primitivo Caballero1

Departamento de Producción Agraria, Universidad Pública de Navarra, Pamplona 31006, Spain.

Address for correspondence: iruiz@unavarra.es

Poster / Bacteria. Tuesday, 10:30. **B-50**

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 suggests 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) as phage display**

Delwar M. Hossain1; Takuya Kotani1; Chiharu Momimoto1; Yuko Harashima2; Ryosuke Sato1

1Tokyo University of Agriculture and Technology, Tokyo 184-8588, Japan. 2Address for correspondence: delwar@cc.tuat.ac.jp

**Bacillus thuringiensis** (Bt) produces the Cry toxin, the insecticidal protein which is induced production at the time of sporulation. This Cry toxin is safe 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**

Venkatassamy Balasubramani1; P. R. Johnston2; Neil Crickmore2

1Department of Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore 641003, India. 2Address for correspondence: neil.crickmore@bt.com

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 / Fungi. Tuesday, 10:30. **F-01**

**Differential UV tolerance amongst spore-cell types of the entomopathogenic fungus Beauveria (Cordyceps) bassiana**

Everton K. K. Fernandes1; Brett H. Kirkland2; Nemat O. Keyhani3; Chad A. Keyser1; Donald W. Roberts1

1Department of Biology, Utah State University, Logan, UT 84322-5305, USA. 2Department of Microbiology and Cell Science, University of Florida, Gainesville, FL 32611, USA. 3Address for correspondence: bernhardt.steinwender@boku.ac.at

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.

Bark beetles are a major threat 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.
The spruce bark beetle, Ips typographus L. (Coleoptera: Scolitidae) is the most important pest for orbital 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 fresh of cultural suspensions of B. bassiana and B. brongniartii (3.2 X 10^2 and 3.2 X 10^3 conidia/ml). The beetles of each variant were incubated with some spruce bark pieces at 20°, 25°, and 30°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 B. bassiana suspension was 79.5- 91.2% and B. brongniartii 33.3-45%.

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 albus), 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 ± 1°C. Ten days after total hatch, larvae were immersed in aqueous conidial suspension (Tween 80 0.001%) at 10^3, 10^4, 10^5 or 10^6 conidia/ml 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 CG406, which caused more than 90% mean mortality. In contrast, isolates UFPE479, UFPE96 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, ESALQ086, ESALQ0747 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.
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% of 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.

**Variability and identification of *Metarhizium* varieties and species based on heat tolerance, cold activity and molecular analysis**

**Everton K. K. Fernandes**; Chad A. Keyser; Drauzio E. N. Rangel; Mark P. Miller; Donald W. Roberts

Department of Biology, Utah State University, Logan, UT 84322-5305, USA.

Address for correspondence: dwroberts@biology.usu.edu

Searches for effective new fungal biological-control agents for insects include not only obtaining new isolates, but also the identification of these species and variety. This is particularly important for *Metarhizium* spp. targeted to locusts/grasshoppers and katydids (all Orthoptera), since *M. anisopliae* var. *anisopliae* (*M.a.*), 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.a.* and *M. frigidum* 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.

**Comparison of new and commercial *Metarhizium* isolates based on multiple traits**

**Chad A. Keyser**; Everton K. K. Fernandes; Drauzio E. N. Rangel; Donald W. Roberts

Department of Biology, Utah State University, Logan, UT 84322-5305, USA.

Address for correspondence: dwroberts@biology.usu.edu

*M. 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 was highly tolerant to 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.
Efficacy of Paecilomyces fumosoroseus blastospores to manage Asian citrus psyllids. Diaporthe 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) sprayed with P. fumosoroseus. Proportions of leaf sections were sprayed as follows: 25%, 50%, 75%, and 100% compared to a yellow tag. 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 25% leaf treatment. Adults began to mycosize 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. Incubated yellow plastic tags may serve as an autodissemination technique for managing psyllid populations in citrus.

Reassessment of vegetative compatibility groups (VCGs) in Japanese isolates of Lecanicillium lecanii (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 aphids more than isolates derived from whitefly. Further, these isolates of V. lecanii were tested for vegetative compatibility by observing heterokaryon formation among complementary nit mutants.

Growth inhibition and revitalization of mycelia of Paecilomyces tenuipes, an entomopathogenic fungus

To establish a stable growth inhibition and culture technique for Paecilomyces tenuipes, a study on the growth control and revitalization of mycelia that were proliferated in papa hosts was performed. All the mycelia that were developed in papa 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, fruits with shapes similar to that of the teleomorph of P. tenuipes developed under certain light conditions; however, perithecial stromata were not formed. The temperature during culturing had no influence on the color and shape of the fruiting bodies.
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 mycoinsecticide. 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=8.998; 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-insoluble 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 photounstabilized 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 photounstabilized titanium dioxide was 74% and 28% compared with 0% for unformulated spores. These results indicate that some formulation compound, particularly water-soluble photounstabilized 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.

In our previous study of bioassays for different developmental stage of Trialeurodes vaporariorum egg by Lecanicillium attenuatum 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 hatching 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, hatching ability 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.

The United Graduate School of Agricultural Sciences, Iwate University, Morioka 0208550, Japan, Toshihiro Watanabe; Masanori Koike 1
Address for correspondence: t-watanabe8605@hotmail.co.jp

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 (2aF43, 43) were used. The aphids killed by ethyl acetate were immersed to conidial suspension(x 10³ 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 10⁶ cfu/cadaver) and at 98% RH also exhibited abundant sporulation(1.04-8.09 x 10⁴ 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 2aF4 produced many conidia on cadaver and the mode of conidiation might be useful to spread the infection.

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 oviposition. More over 2aF43 treatment induced significantly lowest hatchability of them. It can be presumed that V.lecanii 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-14
UV Light protection of Lecanicillium attenuatum with titanium dioxide
Jeong Jun Kim 1, Drauzio E.N. Rangel 2, Donald W. Roberts 3, Dong-jo Choi 4
1Applied Entomology Division, NIAG, 150 Sooin-Ro, Suwon, 441-707, Korea. 2Dept. Biology, Utah State University, 5305 Old Main Hill, Logan UT 84322, USA.
Address for correspondence: jkimm6@hotmail.com

Poster / Fungus. Tuesday, 10:30. 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 3, Masanori Koike 4
1Department of Agro-environmental Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080-8555, Hokkaido, Japan, 2The United Graduate School of Agricultural Sciences, Iwate University, 18-8, Ueda 3-chome, Morioka, Iwate, 020-8550, Japan.
Address for correspondence: koike@obihiro.ac.jp

Verticillium lecanii (Lecanicillium spp.) penetrates into Trialeurodes vaporariorum egg
Daigo Aiuchi 1, Sayaka Horie 2, Masanori Koike 3
1The United Graduate School of Agricultural Science, Iwate University, Morioka 0208550, Japan, 2Department of Agro-environmental Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080-8555, Japan.
Address for correspondence: koike@obihiro.ac.jp

Sporulation of Verticillium lecanii (= Lecanicillium spp.) on cotton aphid cadaver in different humidity conditions
Toshihiro Watanabe 1, Daigo Aiuchi 2, Masanori Koike 3
1Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080-8555, Hokkaido, Japan, 2The United Graduate School of Agricultural Sciences, Iwate University, 18-8, Ueda 3-chome, Morioka, Iwate, 020-8550, Japan.
Address for correspondence: t-watanabe8605@hotmail.co.jp

Poster / Fungus. Tuesday, 10:30. F-15 STU
Lecanicillium spp. (= Verticillium lecanii) penetrate into Trialeurodes vaporariorum egg
Daigo Aiuchi 1, Sayaka Horie 2, Masanori Koike 3
1The United Graduate School of Agricultural Science, Iwate University, Morioka 0208550, Japan, 2Department of Agro-environmental Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080-8555, Japan.
Address for correspondence: koike@obihiro.ac.jp
Field margins composed by noncrop plants are important in annual cropping systems as lettuce, where the 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. Acrystaphion lactucae, Allocorinthus sp., Metopolophium festucae and Ropalosiphum padi killed by Pandora neoaphidis were found on Malva sylvestris, Silybum marianum, Myosotta arvensis and Hordeum marinum. 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.

The entomopathogenic fungus Pandora neoaphidis and aphiphagous 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 analyzed 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. neoaphidis 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.

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.
Isolation and characterization of a photolyase gene from the entomopathogenic fungi Beauveria bassiana.
Lady C. Rosero1; Sandra Valdez1; Luz M. Escobar1; Namer F. Galeano2; Carmenza E. Gongora1
1Department of Entomology, National Centre of Coffee Research CENICAFFE-FNC, PlanAlto, Chinchina, Caldas, Colombia,
2Department of Plant Pathology, CENICAFFE, PlanAlto, Chinchina, Caldas, Colombia.
Address for correspondence: carmenza.gongora@cafedecolombia.com

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 bp. 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 acid binding sites, characteristic of this protein family. A full length cDNA amplified from mRNA extracted from 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.

Identification of transcripts with increased expression during conidiogenesis of the entomopathogenic fungus Metarhizium anisopliae
Everaldo R. Marques1; Sérgio H. Silva1; Donald W. Roberts2; Gilberto U. L. Braga1
1Universidade de São Paulo, Faculdade de Ciências Farmacêuticas, Ribeirão Preto, SP 14040903, Brasil, 2Utah State University, Department of Biology, Logan, UT 843225305, USA.
Address for correspondence: gbraga@fcfrp.usp.br

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.
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._

A survey for entomopathogenic 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 has 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 incidence of the _entomopathogenic fungi was recorded on RPW._ The(KP) _were collected from infected insects, cultured on nutrient agar and characterized both by specific molecular markers (e.g. microsatellites and ITS) and microscopical analysis (SEM, CLSM).

Induction of defense-related genes in banana (_Musa spp._) by endophytic _Fusarium oxysporum_

Pamela Paparu¹; Thomas Dubois²; Daniel Coyne³; Claire Munro⁴; Altus Viljoen³

¹University of Pretoria, 0002 Pretoria, South Africa; ²International Institute of Tropical Agriculture, P.O. Box 7878, Kampala, Uganda; ³University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa.

Address for correspondence: t.dubois@cgiar.org

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 _endophytic strains, particularly against the nematode, have implicated the induction of systemic resistance in banana plants. To identify molecules induced upon entomopathogenic fungi, 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.

Observations of fungal disease in the giant willow aphid (_Tuberolachnus salignus_)

Gudbjorg Aradottir¹;²; Richard Harrington¹; Angela Karp¹; Steve Hanley¹; Ian Shields¹; William Macalpine²; Matilda Collins³; Simon Leather²; Judith Pelli³

¹Rothamsted Research, Harpenden, Hertfordshire; AL5 2QJ, UK; ²Imperial College London, Silwood Park Campus, Ascot, SL5 7PY, UK.

Address for correspondence: gia.aradottir@bbsrc.ac.uk

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.
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 a true Drosophila pathogen. 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 (Bacteria Division) Wednesday, 8:00–10:00**

**Entomopathogenic Bacteria Other than Bacillus**

Symposium. Wednesday, 8:00. 90

*Drosophila* host defence against *Pseudomonas entomophila*

Onya Opota1; Bruno Lemaître1

1Global Health Institute, EPFL, Ecole Polytechnique Federale de Lausanne, School of Life Sciences, GHI, Station 15 (Bâtiment A1), CH-1015 Lausanne Switzerland.

Address for correspondence: onya.opota@epfl.ch

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 a true *Drosophila* pathogen. 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 (Bacteria Division) Wednesday, 8:30–9:30**

**Virulence determinants of *Yersinia entomophaga* MIH96: a genomic perspective.**

Mark R H Hurst1; Regina Shaw2; William G. Farmerie2; Anette Becker3

1AgResearch, Bioprocessing and Biosecurity, Lincoln Research Centre, Canterbury, New Zealand. 2University of Florida, ICBR - Cancer Genetics ResearchComplex-South Wing;PO; Gainesville, FL 32610, USA. 3AgResearch, Applied Biotechnology AgResearch, Invermay, New Zealand.

Address for correspondence: mark.hurst@agresearch.co.nz

A unique insecticidal bacterium designated *Yersinia entomophaga* MIH96 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) rha 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 bacteriopeptide 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 (Bacteria Division) Wednesday, 9:00–10:00**

**Insecticidal toxins from *Photorhabdus*: Comparative genomics and Rapid Virulence Annotation (RVA)**

Richard Hult Hich-Constant1; Stewart Hinchliffe1; Michelle Hares1; Andrea J. Dowling1; Nicholas Waterfield2; Isabella Vlisidou2; Maria Sanchez Contreras2

1University of Exeter in Cornwall, Penryn, TR10 9EZ, UK. 2Department of Biology and Biochemistry, Bath, BA2 7AY, UK.

Address for correspondence: rj222@exeter.ac.uk

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 (Bacteria Division) Wednesday, 9:30–10:30**

**Pathogenesis of *Serratia entomophaga* (Enterobacteriaceae) towards the New Zealand grass grub *Costelytra zealandica*.**

Trevor A. Jackson1; Sean M. Marshall1; Mark R.H. Hurst1; Drion G. Boucas2; Heather S. Gatehouse2; John C. Christeller3

1Biocontrol, Bioprocessing and Biosecurity, AgResearch, Lincoln Research Centre, Canterbury, New Zealand. 2Entomology and Nematology Department, University of Florida, Gainesville, FL32611, USA. 3Horticulture and Food Research Institute, Palmerston North, New Zealand.

Address for correspondence: trevor.jackson@agresearch.co.nz

*Serratia* spp. (Enterobacteriaceae) are commonly isolated from grassland soils. In New Zealand, isolates of *S. proteamaculans* and *S. entomophaga* 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 grass 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

Miroslav Hylíš1

1Faculty of Science, Charles University, Vinicna 7, Prague, Czech Republic.

Address for correspondence: mirekhylis@volny.cz

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, Paraepseptum and Zelenkaia are host-specific species 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, Simulidae). 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

Judith E. Smith1; Qui Yang2; Raviil M. Kamaltynov2; Dmitry Y. Sherbakov2

1Leeds University, IICB, Miall Building, Faculty of Biological Sciences, Leeds LS2 9JT, UK, 2Institute of Limnology, Siberian Branch of Russian Academy of Sciences, P.O.Box 4199, 664033 Irkutsk, Russia.

Address for correspondence: j.e.smith@leeds.ac.uk

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

Remi A. Wattier1; Karolina Bacela1; Thierry Rigaud1

1Equipe Ecologie Evolutive, UMR CNRS 5561 Biogéosciences, Université de Bourgogne, 6 bd Gabriel, Dijon, Burgundy, 21000, France.

Address for correspondence: remi.wattier@u-bourgogne.fr

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

Ellen Decaestecker1 1K.U.Leuven - Campus Kortrijk, Belgium.

Address for correspondence: ellen.decaestecker@kuleuven-kortrijk.be

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 Ambyl ospora camposi (Microsporida: Amblyosporidae) in *Culex renatoi* (Diptera: Culicidae) and Paracyclops fimbriatus fimbriatus (Copeoda: Cyclopidae) in a bromiumal habitat**

Victoria Miceli1; James J. Becnel1; Gerardo A. Martí1; Maria C. Tranchida1; Juan J. García2

1Centro de Estudios Parasitológicos y de Vectores-CEPAVE (UNLP-CONICET), 2 N° 584, La Plata, Argentina, 2Department of Agriculture, Agriculture Research Service, Center for Medical, Agricultural and Veterinary Entomology, 1600 SW 23rd Drive, Gainesville, FL 32608, USA.

Address for correspondence: james.becnel@ars.usda.gov

The epizootiology of *Ambylospora 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 caberae* plants were sampled monthly and the prevalence of *A. camposi* in *P. f. fimbriatus* and *C. renatoi* populations was determined. The monthly prevalence rates of meiospore infections in *C. 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 x 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 x 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 microsporidian has adapted to the ecological parameters of its hosts and the specialized habitat where they are found in nature to ensure long term survival.
selectable marker (phosphonothricin 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**

Eudes de Crecy; Nemat O. Keyhani

\textsuperscript{1}Evolutage LLC, 2153 SE Hawthorne Road, \#15 Gainesville, FL, 32641, USA, \textsuperscript{2}Microbiology and Cell Science, University of Florida, Bldg 981, Museum Rd. Gainesville, FL 32611, USA.

Address for correspondence: keyhani@ufl.edu

\textit{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**

Galinia Gindin; Dana Ment; Asael Rot; Itamar Glazer; Michael Samish

\textsuperscript{1}The Volcani Center, (ARO),Department of Entomology and Nematology, Bet Dagan, 50250, Israel, \textsuperscript{2}Kimron Veterinary Institute, Department of Parasitology, Bet Dagan, 50250, Israel.

Address for correspondence: samishm@int.gov.il

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. \textit{anisopliae} conidia adhering to tick cuticle is a dose-response process that is directly correlated with tick mortality. Conversely, the amount of conidia of the low virulent isolate \( M. \) an var. \textit{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. \textit{an} appressorium, largely depends on the tick susceptibility to the fungus. The germination of \( M. \) an. \textit{acridum} 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**

Sérgio H. Silva; Bruno H. R. Barros; Everaldo R. Marques; Ana Patrícia Yatsuuda; Donald W. Roberts; Gilberto U. L. Braga

\textsuperscript{1}Universidade de São Paulo, Faculdade de Ciências Farmacêuticas, Ribeirão Preto SP 14040903 Brazil, Department of Biology, Utah State University, Logan, UT 84322-5305, USA.

Address for correspondence: gbraga@fcrp.usp.br

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. \textit{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-phosphogluconate dehydrogenase, the allergen Alt A 7, a predicted vacuolar protease A, and a predicted mitochondrial peroxiredoxin. 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**

Javier G. Mantilla; Sandra M. Idarraga; Alvaro L. Gaitán; Carmenza E. Gonzora

\textsuperscript{1}Department of Entomology, National Centre of Coffee Research CENICA菲E-FNC, PlanAlto, Chinchiná, Caldas, Colombia, \textsuperscript{2}Department of Plant Pathology, National Centre of Coffee Research CENICA菲E-FNC, PlanAlto, Chinchiná, Caldas, Colombia.

Address for correspondence: carmenza.gonzora@cafede.colombia.com

To understand the infection process of the entomopathogen \( Beauveria \) bassiana on the Coffee Berry Borer (CBB) \textit{Hypothenemus hampei}, full length cDNAs were obtained from mycelium of the strain BB9205 growing for 4h in a minimal medium plus 10% v/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

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\textsuperscript{1}Sérgio H. Silva; Bruno H. R. Barros; Everaldo R. Marques; Ana Patrícia Yatsuuda; Donald W. Roberts; Gilberto U. L. Braga

\textsuperscript{1}Universidade de São Paulo, Faculdade de Ciências Farmacêuticas, Ribeirão Preto SP 14040903 Brazil, Department of Biology, Utah State University, Logan, UT 84322-5305, USA.

Address for correspondence: gbraga@fcrp.usp.br

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. \textit{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-phosphogluconate dehydrogenase, the allergen Alt A 7, a predicted vacuolar protease A, and a predicted mitochondrial peroxiredoxin. 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**

Javier G. Mantilla; Sandra M. Idarraga; Alvaro L. Gaitán; Carmenza E. Gonzora

\textsuperscript{1}Department of Entomology, National Centre of Coffee Research CENICA菲E-FNC, PlanAlto, Chinchiná, Caldas, Colombia, \textsuperscript{2}Department of Plant Pathology, National Centre of Coffee Research CENICA菲E-FNC, PlanAlto, Chinchiná, Caldas, Colombia.

Address for correspondence: carmenza.gonzora@cafede.colombia.com

To understand the infection process of the entomopathogen \( Beauveria \) bassiana on the Coffee Berry Borer (CBB) \textit{Hypothenemus hampei}, full length cDNAs were obtained from mycelium of the strain BB9205 growing for 4h in a minimal medium plus 10% v/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 monoxygenases**

Nicolas Pedrini1;2; Patricia Juárez1; Nemat O. Keyhani1

1 Instituto de Investigaciones Bioquímicas de La Plata (CCT CONICET-UNLP), Facultad de Ciencias Médicas, Calles 60 y 120, La Plata, 1900, Argentina. 2Microbiology and Cell Science, University of Florida, Bldg 981, Museum Rd. Gainesville, FL 32611, USA.

Address for correspondence: nicopedrini@yahoo.com

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?**

Almudena Ortiz-Uquizá1; Laura Grieve-Miranda1; Cándido Santiago-Alvarez2; Enrique Quesada-Moraga1

1 University of Córdoba, Campus de Rabanales, Building C4. 2"Celestino Mutis" 14071 Córdoba, Spain.

Address for correspondence: g82ortuz@uco.es

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 01/110-su, EABb 01/103-su, EABb 01/110-su) grown on 4 different media; Malt Extract Agar (MEA), Rice (R), Sabouraud 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 01/110-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**

Michael W. Lewis1; Ines V. Robalino1; Nemat O. Keyhani1

1 Microbiology and Cell Science, University of Florida, Bldg 981, Museum Rd. Gainesville, FL 32611, USA.

Address for correspondence: keyhani@ufl.edu

*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, lactruclerin B, and cytochalasin D were examined in the gernlings. 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.
Deletion of the egt gene reduces within-host competitive fitness

Mark Zwan1; Wopke van der Werf2; Monique van Oers3; Lia Hemerik3; Jan van der Lent1; Arjan G. M. de Visser1; Just M. Vlak3

1Laboratory of Virology, Wageningen University, The Netherlands, 2Crop and Weed Ecology, Wageningen University, The Netherlands, 3Biometris, Wageningen University, The Netherlands. Address for correspondence: jenny.cory@algomau.ca

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 resulting 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.

Characterization of climbing behavior gene in recombinant baculoviruses

Matthew R. Gardner1; James M. Slavicek2; Scott M. Geib3; Kelli Hoover4

1Pennsylvania State University, 501 ASI, University Park PA 16802, USA, 2USDA Forest Service, 359 Main Road, Delaware, OH 43015, USA. Address for correspondence: mrg257@psu.edu

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 (ecdysteroid 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 (7HS) did not. Amplification of the egt gene was successful for 323c, but not 7HS. Restriction digests showed a deletion in the egt gene in 7HS. 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 7HS. These results confirm observations that insects infected with LdNPV lacking intact egt gene do not seek elevated positions prior to death.

Chrysodeixis chalcites nucleopolyhedrovirus encodes an active DNA photolyase

Monique M. van Oers1; Margit H. Lampen1; Monika I. Bajek2; Fang Xue3; Just M. Vlak4

1Laboratory of Virology, Wageningen University, Binnenhaven 11, 6709PD Wageningen, the Netherlands, 2Department of Cell Biology and Genetics, Erasmus University Medical Centre, Dr. Molenvliet 50, 3015 GE Rotterdam, the Netherlands. Address for correspondence: monique.vanoers@wur.nl

DNA photolyase gene (phr) encode photoreactive enzymes, which are involved in the repair of UV-damaged DNA. Cyclobutane pyrimidine dimer (CPD) specific photolyase gene are present in nucleopolyhedroviruses isolated from Chrysodeixis chalcites (ChchNPV) and Trichoplusia ni (TnSNPV) insects belonging to the Plusiineae 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.

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-phr2 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. In vitro, recombinant Cc-phr2 photolyase specifically bound F0 (8-hydroxy-7,8-didemethyl-5-deazariboflavine), 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**

James R. McNeil\(^1\), Diana Cox-Foster\(^2\), Lauren Ellis\(^3\), Kelli Hoover\(^1\)

\(^1\)Penn State University, 501 ASI, University Park, PA, 16802, USA.

Address for correspondence: jrm418@psu.edu

Gypsy moth (Lymantria dispar) larvae show intratradial 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**

Nor Chejanovsky\(^1\), Haddassah Rivkin\(^1\), Irit Ornan\(^1\)

\(^1\)Entomology Department, Institute of Plant Protection, The Volcani Center, POB 6 Bet Dagan, 50250, Israel.

Address for correspondence: ninar@volcani.agri.gov.il

*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 *cyt-motif* and *vanlyn* 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. **

**An AcMNPV vfgf knockout mutant exhibits a defect in systemic infection of Trichoplusia ni larvae**

John C. Means\(^1\), A. Lorena Passarelli\(^1\)

\(^1\)Division of Biology, Kansas State University, 116 Ackert Hall, Manhattan KS 66506, USA.

Address for correspondence: ipassar@ksu.edu

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 AcMNPV *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 AcMNPV *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 AcMNPV 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**

Peters Are\(^1\) e-Nema, Raisdorf, Germany.

Address for correspondence: a.peters@e-nema.de

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. Aboveground applications in non-protected crops (apples) have recently been established.
Cadaver application

Claudia Dolinski1; Edwin E. Lewis2; David Shapiro-Ilan3

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 (IJ) 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.

Above ground and cryptic habitats application

Richard Glass1; Keith F. Walters3

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.

Contributed paper. Wednesday, 10:30. 119

A novel gene cluster encoding an insect toxin in plant-associated strains of Pseudomonas fluorescens

Maria Pechy-Tarr1; Denny J. Bruck2; Monika Maurhofer3; Esther Fischer4; Christelle Voge1; Jurg Grunder4; Joyce E. Loper2; Christoph Keel1

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 exoproteins that are toxic to 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-plantizing bacteria.
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 2, Alain Givaudan 1, Eric Oswald 1, Jean-Michel Escoubas 1, Robert Zumbühl 1

1 Université Montpellier 2, IRNA EMIP UMR 1313, Place Eugène Bataillon bât 24 CC 34059 Montpellier cedex 05, 34095, UM 1225, INRA-ENV'T, 23 Chemin des Capelles, BP 87614, 31000 Toulouse, France.

Address for correspondence: cvarela@univ-montp2.fr

*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 entomopathogenic 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* strains. 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 lambdoid 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*

Ziad Abi Khattar 1, Anne Lanois 1, Sylvie Pagès 1, Mireille Kallassy 1, Sophie Gaudriault 1, Alain Givaudan 1

1 Université Montpellier II, IRNA EMIP UMR 1313, Place Eugène Bataillon, Bat 24 CC 34-34059 Montpellier cedex 05, France.

Address for correspondence: zakibkat@univ-montp2.fr

*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 (*ipzM*) 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 cephalosporin A. *MdtC* is a transmembrane homomultimer of the multidrug transporter MdtABC. *BaeS* is the sensor of the two component regulatory system *baeS* which activates several drugs efflux pumps in bacteria. Our findings suggest that the *msbB* gene and the *mdtABC*/*baeS* operons are potential candidates involved in *P. luminescens* resistance to *Spodoptera*'s AMPs and eventually in insect pathogenicity.

Contributed paper. Wednesday, 11:30. **123 STU**
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 V197A, K199A, Q200A, K201A, F204A and T206A. Replacement substitutions in the beta6-beta7 loop were performed (S194A, Q200A and F204A). 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.

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 greenhouse 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.
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 45° angle to the ground. The sprayer was alternately 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 85° angle to the ground. The sprayer was operated at a ground speed of 4.8 km/h. Hollow-cone nozzles of 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 10⁶ 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/mm², 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.

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 LD₅₀. Our results demonstrate the importance of entomopathogenic fungi as an agent microbial control with respect to the production conditions.
Effect of preying on *Metarhizium anisopliae* - infected onion thrips larvae on some behavioral parameters of *Orius albidipennis*

Hamid-Reza Pourian 1; Reza Talaei-Hassanlou 2; Reyhaneh Ezzati-Tabrizi 1; Aziz Kharazi-Pakdel 1

1 Campus of Agriculture and Natural Resources, University of Tehran, College Street, Karaj 31587-11167, Iran.

Address for correspondence: ralaei@ut.ac.ir

The general predator *Orius albidipennis* and entomopathogenic fungus *Metarhizium anisopliae* are the 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 1x10^3, 2x10^3 and 2x10^3 conidia/ml as nearly LC50, LC30 and LC75 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.

Assessing potential effects of the *Beauveria brongniartii* biological control agent on fungal community structure in soil microcosms

Juerg Enkerli 1; Kaspar Schwarzenbach 1; Franco Widmer 1

1 Agroscope Reckenholz-Taenikon Research Station ART, Reckenholzstrasse 191, 8046 Zurich, Switzerland.

Address for correspondence: juerg.enkerli@art.admin.ch

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.

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, Mansfield Road OX1 3SR, UK.

Address for correspondence: rmurri@ceh.ac.uk

Baculoviruses are recognized for causing overt 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 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.
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.

**Vertical transmission and persistent infection of NPVs in Eastern Spruce Budworm**

Elizabeth M. Kemp1,2; David T. Woodward1; Jenny S. Cory1,2
1Algoma University College, 1520 Queen St E., Sault Ste Marie, ON, P6A 2G5, Canada; 2Great Lakes Forestry Centre, Canadian Forest Service, Sault Ste Marie, ON, P6A 2E5, Canada

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, CIMNPV and CIDEFNPV. The latter is not infectious to *C. fumiferana* per os but is involved in a symbiotic relationship with CIMNPV. Both isolates appear capable of persistently infecting the host. We have attempted to establish a persistent baculovirus infection with a genetically modified CIMNPV 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 LD90 of recombinant CIMNPV; 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 parents had survived virus challenge but not from unchallenged parents.

**Aggregation and infection risk in Lepidoptera**

Joanna C. McTigue1, Steve M. Sait2, Rosie S. Hails3
1Centre for Ecology and Hydrology, Mansfield Road, Oxford, OX1 3SR, UK; 2University of Leeds, Leeds, LS2 9JT, UK

Address for correspondence: jobro@ceh.ac.uk

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*.
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 ST₅₀ (using an LD₅₀ dose) of 37.0 days in neonates. AdorNPV is fast-killing with an ST₅₀ (using an LD₅₀ 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 LD₅₀ dose of AdorGV, an LD₅₀ dose of AdorNPV, and 50:50, 25:75 and 75:25 mixes of LD₅₀ 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. 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.

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.

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.
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
S. Patricia Stock1 University of Arizona, USA.
Address for correspondence: spstock@ag.arizona.edu

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
Dominique Yue1, Anne Fünfhäus1, Ainura Ashiraliyev1,
Elke Genersch1
1Institute for Bee Research, Friedrich-Engels-Str. 32, 16540 Hohen Neuendorf, Germany.
Address for correspondence: elke.genersch@rz.hu-berlin.de

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 fluorescent 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
Mark J.F. Brown1 University of Dublin Trinity College, Ireland.
Address for correspondence: mabrown@tcd.ie

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
Joachim R. de Miranda1, Ingemar Fries1
1Queen’s University Belfast, Biological Sciences, MBC, 97 Lisburn road, Belfast, BT9 7BL, Northern Ireland, Swedish University of Agricultural Sciences, Department of Ecology, POBox 7044, Uppsala, 750-07, Sweden.
Address for correspondence: j.rodrigues@qub.ac.uk

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
Rosalind James1 USDA, ARS, Logan, UT, USA.
Address for correspondence: Rosalind.James@ARS.USDA.GOV

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, epizoic fungus is 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
Denis L. Anderson1
1CSIRO Entomology, P.O. Box 1700, Canberra ACT 2601, Australia.
Address for correspondence: Denis.Anderson@csiro.au

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. 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...
Kees van Frankenhuyzen1; Carl Nystrom1
1Natural Resources Canada, Canadian Forest Service, Great Lakes Forestry Centre, 1219 Queen street East, Sault Ste. Marie, Ontario, P6A 2E5, Canada.
Address for correspondence: kvanfran@nrcan.gc.ca

Many data on insecticidal activity of delta-endotoxins from B. thuringiensis have been published since cry genes were first cloned. The Bt toxin specificity database1, 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 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 LD50s are in the 0.5 to 1.5 ng toxin per cm2 range (Cry1A toxins against a variety of species). Similar analyses are in progress for assays conducted with Coleoptera and Diptera. (http://www.gifc.forestry.ca/bacillus)

Contributed paper. Wednesday, 14:15. 152

Gut flora not required for pathogenicity in Bacillus thuringiensis infecting diamondback moth
Ben Raymond1; Michael B. Bonsall1
1Dept of Zoology, Oxford University, South Parks Rd, Oxford, OX1 3PS, UK.
Address for correspondence: benjamin.raymond@zoo.ox.ac.uk

Recent experimental work has indicated that Bacillus thuringiensis (Bt) cannot exploit aecitic hosts experimentally cued 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 aecitic insects or (2) Bt is sensitive to the antibiotic treatments used to make insects aecitic. We developed methods for producing aecitic 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 (riR) Bt A second experiment tested whether diverse strains (wild types; biopesticide isolates and passed riR strains) could grow in rifampicin treated larvae or in untreated aecitic larvae. Rifampicin treated insects only reduced larval mortality for rifampicin sensitive Bt but not riR Bt. All strains could grow in untreated aecitic 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
Kees van Frankenhuyzen1; Yuehong Liu1
1Natural Resources Canada, Canadian Forest Service, Great Lakes Forestry Centre, 1219 Queen street East, Sault Ste. Marie, Ontario, P6A 2E5, Canada.
Address for correspondence: kvanfran@nrcan.gc.ca

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 septicaemia 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 >109cfu 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 (~106cfu/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.

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**WEDNESDAY PM**

**154 STU**

**Distinct changes in immune system are associated with Bt exposure in Bt-resistant and Bt-susceptible Trichoplusia ni colonies.**

Jerry D. Ericsson1; Aldia F. Jannaat2; Richard M. Plunkett2; Judith H. Myers2; Carl Lowenberger1

1Department of Biological Sciences, Simon Fraser University, 8888 University Drive, Burnaby, BC, V5A1S6, Canada;
2Biology Department, University College of the Fraser Valley, 6270 University Blvd., Vancouver, BC, V6T1Z4, Canada.

Address for correspondence: jericsson@sfu.ca

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*.

**Characterization of intracellular response in mosquitoes to Bacillus thuringiensis Cry1Aa toxin.**

Ángeles Cancino-Rodríguez1, Roberto Villaseñor1, Mario Soberón1, Sergio Encarnación1, Humberto Lanz2, Ivonne Castro2, Juan Luis Jurat-Fuentes3 and Alejandra Bravo1.

1Instituto de Biotecnología y Agroalimentación. UNAM, Av. Universidad #2001, C.P. 62210, Cuernavaca, Morelos, Mexico; 2Centro de Ciencias Genómicas UNAM, Av. Universidad #2001, C.P. 62210, Cuernavaca, Morelos, Mexico; 3Centro de Ciencias Genómicas UNAM, Av. Universidad #2001, C.P. 62210, Cuernavaca, Morelos, Mexico.

Address for correspondence: slege@ibt.unam.mx

Dengue and malaria are human diseases produced by arboviruses 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 *Bti* toxins in *Caenohabditis elegans*. Gene silencing of p38 has been previously shown to increase the specific susceptibility of *C. elegans* to *Bti* 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.

**Kinetics of microbial degradation and chemical fixation of Cry 1Aa Bt toxin in various soils.**

Nordine Helassa1; Arj McCharek1, Gabrielle Daun1; Sylvie Nounville1; Philippe Déjardin1; Hervé Quisquampoix1; Siobhan Staunton1

1INRA - Biogéochimie du Sol et de la Rhizosphère, 2 place Viala, 34060 Montpellier Cedex, France; 2Faculté des Sciences de Tunis - Laboratoire de Biochimie et de Biotechnologie, Campus universitaire, 2092 El-Manar II, Tunisie; 3CNRS - Laboratoire de Dynamique, Interactions et Réactivité, 2 rue Henry Dunant, 94320 Thiais, France; 4CNRS - Institut Européen des Membranes, 2 place Eugène bataillon, 34095 Montpellier Cedex, France.

Address for correspondence: helassa@supagro.inra.fr

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, 14:45. 156 STU**

**157 STU**

**The ger genes of pBtoxis are responsible for the alkaline-activation of germination in Bacillus thuringiensis subsp. israelensis.**

Mostafa Abdoorrahemi1; Colin Berry1

1Cardiff School of Biosciences, Cardiff University, Museum Avenue, Cardiff CF10 3US, UK.

Address for correspondence: Berry@cf.ac.uk

*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**

Konasale J. Anilkumar1; William J. Moar1

1Auburn University, 301 Funchess Hall, Auburn, AL 36849 USA.

Address for correspondence: anilkkj@auburn.edu

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 NB 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 NB 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.

**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?**

Aki Fujimoto1; Gulumser Cohanoglu2; Ed E. Lewis1; Harry K. Kaya3

1Kumiai Chemical Industry Co. 3360 Kamo, Kikugawa-shi, Shizuoka, 4390031, Japan, 2Hacettepe University, Faculty of Science, 06800- Bayepe/Ankara, Turkey, 3University of California, One Shields Avenue, Davis, CA 95616, USA.

Address for correspondence: hkkaya@ucdavis.edu

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, 14:30. **161 STU**

**Habitat preferences of nictating nematodes**

Laura M. Kruitbos1; Stuart Heritage2; Mike J. Wilson1

1Institute of Biological & Environmental Sciences, Cruickshank Building, University of Aberdeen, St Machar Drive, Aberdeeen, AB24 3UU, UK, 2Forest Research, Roslin, Midlothian, EH25 9SY, UK.

Address for correspondence: l.kruitbos@abdn.ac.uk

The nictation behaviour of entomopathogenic nematodes; Steinernema 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 hermaphroditia 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**

John Mukuka1; Olaf Strauch2; Ralf Udo-Ehlers1

1Christian-Albrechts-Kiel University, Hermann-Rodewald-Str.9, Germany. 2Research Institute for Agriculture, Gyula, Hungary.

Address for correspondence: john.mukuka@biotec.uni-kiel.de

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 ge-climatic regions. Dehydrating conditions were produced by treating DJs with non-ionic polymer polyethylene glycol 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 (α ≤ 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
Ayako Hiroa; Ralf Udo Ehlers
1Institute for Phytopathology, University Kiel, Hermann-Rodewald-Str. 9, 24118, Kiel, Germany.
Address for correspondence: ayako.hirao@biotec.uni-kiel.de

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
Mehmet Karagoz; Selcuk Hazir; Ibrahim Cakmak; Baris Gulcu; Harry K. Kaya
1Department of Plant Protection, University of Adnan Menderes, Kocarli, Aydin, Turkey, 2Department of Biology, University of Adnan Menderes, Ay tepe Kampusu 09010, Aydin, Turkey, 3Department of Nematology, University of California, Davis, CA, 95616, USA.
Address for correspondence: selcuk.hazir@gmail.com

Sancassania sp. (Acar: 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 and the nematodes did not infect the mites.

CONTRIBUTED PAPERS Wednesday, 14:00-16:00

VIRUSES 5

Baculovirus IE2 forms nuclear bodies in the nucleus and enhances CMV promoter expression in mammalian cells
Catherine Y. Y. Liu; Chia-Hung Wang; Wen-Kai Hsiao; Yu-Chan Chao
1Institute of Molecular Biology, Academia Sinica, No. 128, Sec. 2, Academia Road, Nankang, Taipei 115, Taiwan, ROC.
Address for correspondence: mbycchao@gate.sinica.edu.tw

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 gene 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.
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, P55 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.

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 LEF-3 function extends beyond nuclear localization and transport of P143, multiple NLS sequences to enhance replication efficiency is also being considered. A similar approach was used within aa 2-125 to investigate LEF-3 interaction. Preliminary results show that deleting Gly552 in P143 inhibits interactions 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.

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 CMNPV (C) 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 1-167 to investigate LEF-3 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.

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.
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 the DNA pol. Western analysis showed that a 100 kDa protein is 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 the DNA pol. Western analysis showed that a 100 kDa protein is 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 the DNA pol.

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.

### POSTERS – 2

**MICROBIAL CONTROL**

**WEDNESDAY PM**

**Contribution paper. Wednesday, 15:45. 172**

**SV40 polyadenylation (pA) signal increases transcription but reduces protein production in baculovirus expression vector system**

Craig P. Seaborn; Tamer Z. Salem; Colin M. Turney; Jianli Xue; Xiao-Wen Cheng.

Department of Microbiology, Miami University, 32 Pearson Hall, Oxford, Ohio 45056, USA.

Address for correspondence: chengx@muohio.edu

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 poly(A) promoter with and without SV40 poly(A) was inserted into the polyhedrin, edcsyteroid 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 PM**

**Contribution paper. Wednesday, 15:15. 170 STU**

**Structural and functional analysis of the Chilo iridescent virus DNA polymerase promoter**

Ikhla Agah Ince; Remziye Nalçacoglu; Zihni Demirbag; Just M. Vlak; Monique M. van Oers.

1\ Wageningen UR, Laboratory of Virology, Binnenhaven 11, Building 504, 6709PD, Wageningen, The Netherlands. 2\ Arts and Sciences Faculty, Department of Biology, Karadeniz Technical University, 61080, Trabzon, Turkey.

Address for correspondence: agah.ince@wur.nl

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 the DNA pol. Western analysis showed that a 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 the DNA pol.

**Contribution paper. Wednesday, 15:30. 171 STU**

**Suppression of AcMNPV gene expression in mammalian cells**

Ryosuke Fujita; Shinichiro Asano; Ken Sahara; Hisanori Bando.

1\ Research Faculty of Agriculture, Hokkaido University, N9W9, Sapporo, Hokkaido, Japan.

Address for correspondence: rfujiita@lab.nig.ac.jp

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.

**Production and evaluation of mosquitoicidal efficacy of Bacillus thuringiensis subsp. israelensis based formulations in Vietnam**

Binh D. Ngo; Tuan D. Nguyen; Hai T. Trinh.

1\ Institute of Biotechnology, Vietnamese Academy of Science and Technology, 18 Hoang Quoc Viet Road, Hanoi, Vietnam.

Address for correspondence: binh.gen@ibt.ac.vn

*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 nm 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, 94.6 and 100 % respectively. In experiment for long-term effect of products, the formulations CT1, 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.
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* cry1B, the chiA gene of *S. marcescens* ATCC990 was transformed separately into respective bacterium. Extracellular chitinase of transformants was 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.cry1B as compared to TBs.1214. The appearance of clear zone on 1% chitin agar plate produced by transformants TBs.S3 and TBt.cry1B 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.cry1B, TBs.S3 exhibited higher activity against 16 plant fungal pathogens tested. Transformant TBs.S3 exhibited higher antifungal activity against *Sclerotinia rolfsii* and *Pythium myriotylum* than its host (*B. subtilis* S3). Transformant TBt.cry1B exhibited higher antifungal activity against *P. myriotylum* than its host (*B. thuringiensis* cry1B).

**Construction of a recombinant Bacillus subtilis strain as an integrated control agent being able to control to plant diseases and insect pests**

Jong Yul Roh1; Jae Young Choi; Yong Wang; Jin Cheol Kim; Yeon Ho Je2

1Department of Agricultural Biotechnology, College of Agriculture and Life Sciences, Seoul National University, Seoul 151-742, Korea. 2Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 151-742, Korea. 3Chemical Biotechnology Research Center, Korea Research Institute of Chemical Technology, Daejeon 305-343, Korea. Address for correspondence: brus@snu.ac.kr

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 (pH71K-1Ac). The plasmid, pH71K-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 approx. 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.
The incorporation of certain stilbene optical brighteners into virus-based formulations has been demonstrated to increase viral pathogenicity (as indicated by reduced LD/LC50 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 and Helicoverpa armigera single nucleopolyhedrovirus

Maria A. Ibarguchi1; Alejandra Bernal1; Delia Muñoz1; Iligo Ruiz de Escudero1; Primitivo Caballero1

1Instituto de Agrobiotecnología, CSIC, Universidad Pública de Navarra, Gobierno de Navarra, 31006 Mutriku Baja, Spain.

Address for correspondence: angeles.ibarguchi@unavarra.es

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

The experiment was carried out at the Laboratory of Entomology of the Centro de Ciências Agrárias da Universidade Federal do Espírito Santo (CCA-UFS) to verify the susceptibility of Trichoplusia ni first instar larvae to 60 isolates of the entomopathogenic bacterium Bacillus thuringiensis. A suspension with 3 x 10^3 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-04**

Susceptibility of Trichoplusia ni (Lepidoptera: Noctuidae) to Bacillus thuringiensis

Ricardo A. Polanczyk1; Eduardo D. Grecco1; Dirceu Pratiollo1; Cláudio R. Franco1; Luiz Flávio V. Silva1

1Centro de Ciências Agrárias - Universidade Federal do Espírito Santo, Alto Universitário S/N, Alegre-ES, 29500-000, Brasil.

Address for correspondence: rapolanc@yahoo.com.br

The European pine sawfly Neodiprion sertifer (Geoffr.), and pine looper Bupalus piniarius L. are ready made components of IPM systems that will not pose a threat to applicators or the environment. Control of several orchard pests 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 organic growers with interest also shown by conventional growers.

Poster / Microbial Control. Wednesday, 16:30. **MC-08**

Searching for pathogens to control stored product mites (Acari: Acaridida)

Iñigo Ruiz de Escudero1; Tomás Erban, Crop Research Institute, Drnovska, 507, Prague, CZ 16108, Czechia.

Address for correspondence: hubert@uvuv.cz

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 infestations 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 acarid 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 bulbannillii and 91% similarity to Photorhabdus temperata. In midgut cells of stored product mites, the unidentifed icoshedral 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

David Shapiro-Ilan1; Lawrence A. Lacey2

1USDA-ARS, S.E. Fruit and Tree Nut Research Laboratory, 21 Dunbar Rd., Byron, GA, 31008, USA. 2USDA-ARS, Yakima Agriculture Research Laboratory, Wapato, WA, 98908, USA.

Address for correspondence: David.Shapiro@ars.usda.gov

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**

C. Chkhubianishvili 1, I. Malania 2, M. Kakhadze 1, N. Mikaya 1
1Department of Biological Control, Kanchaveli L. Institute of Plant Protection, Tbilisi 0162, Georgia.

Address for correspondence: cisia@ymail.ge

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-GBE2-3337-TO-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 nematode, 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**

Steven P. Arthurs 1, Lance Osborne 1
1Mid-Florida Research & Education Center, 2725 S. Binion Road, Apopka, FL 32703, USA.

Address for correspondence: stevenarthurs@hotmail.com

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**

Nicolas Pedrini 1, Carolina Cambiasso 1, Patricia Juarez 1
1Instituto de Investigaciones Bioquímicas de La Plata (CCT CONICET-UNLP), Facultad de Ciencias Médicas, Calles 60 y 120, La Plata, 1900, Argentina.

Address for correspondence: nicopedrini@yahoo.com

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 (104, 105, and 106 conidia/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 bassiana conidia on their viability and pathogenicity against the onion thrips, Thrips tabaci**

Reyhaneh Ezzati-Tabrizi 1, Reza Talaei-Hassanlou 1, Aziz Kharazi-Pakdel 1, Khalil Talebi 1
1University of Tehran, Campus of Agriculture and Natural Resources, College Street, Karaj 31587-1167, Iran.

Address for correspondence: rtlalei@ut.ac.ir

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 104 conidia/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 (MgCl2, NH4H2PO4, KH2PO4, MgSO4 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.
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.

The red palm weevil *Rhynchophorus ferrugineus* (RPW) is a serious pest of date palm (*Phoenix dactylifera*) and other Palmaeae in Spain and other Mediterranean countries. Recent outbreaks of RPW in Spain prompted 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 through 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. LT50 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.

The two-spotted spider mite, *Tetranychus urticae* is the most important pest to Brazilian papaya, especially at Espirito Santo State. The control of this pest by chemicals has increased the environmental problems and pollution and besides this it has been observed higher rates of resistance evolution. The entomopathogenic fungi *Beauveria bassiana* and *Cladosporium cladosporioides* were assayed against this pest as an alternative to chemical control. Conidial suspensions (107 conidia/mL) were prepared and offered to ten females in *Canavalia ensiformis* 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 promising biological control agents for the control of *T. urticae* in Brazilian papaya.
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 Morocco 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.

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 (1x10⁴ 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 (1x10⁵ 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.

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* (i.e., 60) suspension containing 10⁷ conidia/ml formulated in sterile distilled water, emulsifiable 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 aequous 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.

The virulence of five isolates of the entomopathogenic fungus *Metarhizium* were evaluated against *Zabrotes subfaciatus* (Boheman) (Coleoptera: Bruchidae) in Ethiopia. Four native isolates, META-B, META-D, PPRC-6, PPRC-29, and one standard isolate, ICPE-30 were applied at different concentrations of conidia/ml. The experimental bruchids were treated by spraying 1ml of each fungal concentration. All the isolates tested were found to be virulent at different magnitudes based on level of conidial concentrations. Among the Metarhizium isolates, META-D and ICPE-30 were proved to be best performing in their virulence compared with other isolates tested. In most post treatment 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 approach to chemical insecticides against one of the major sorage insect pests, the Mexican bean bruchid. Discussions in this presentaion are expected to to contribute towards expericence sharig in advancing the development and application of myco-insecticides for stored product pests in general.
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 emerged from unmatched heads (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 define the host specificity of V. invictae and indicated that V. invictae will not directly interfere with P. obtusus parasitism.

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 parasitizing the stink bug Rhyphigaster 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 Rhyynchota. 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.
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 carposcapae applied to soil as infected insect cadavers. We quantified S. carposcapae dispersal by bioassay of soil samples collected at distances up to 3 m away from the application point within and between crop and refuge habitats. Detection of S. carposcapae 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 7.5 cm/day. In 2005, soil moisture had the largest effect on dispersal with S. carposcapae 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. carposcapae is not necessarily dependent on plant diversity, but may respond to variation in factors associated with overall plant density, and subsequently, soil moisture.

The insect parasitic nematode *Thripinema fuscum* is a key regulator of *Frankliniella fuscus* 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. fuscus* to spread Tomato spotted wilt virus. The potential of *T. fuscum* to act as a biological control agent of *F. fuscus* 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. fuscus* tissues affected by *Thripinema* invasion and replication were recorded and a possible explanation of the cause provided.

Investigations of toxicity of simpler molecules based on the epoxyalcohol fragment of azadirachtin have revealed insecticidal activity on *Galleria mellonella* L. larvae. The epoxy-alcohols 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 occurred mainly on *P. pacificus* 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.

**SUSCEPTIBILITY OF THE COLORADO POTATO BEETLE TO THE NEMATODE P. PACIFICUS**

Andreas M. Weller, Ralf J. Sommer

1Department for Evolutionary Biology, Max Planck Institute for Developmental Biology, Am Klopferspitz, 72775 Tübingen, Germany.

Address for correspondence: andreas.weller@tuebingen.mpg.de

**OTHER**

**TOXICITY OF AZADIRACHTIN AND SOME OF ITS MOLECULE ANALOGUES**

Carole Charbonneau, Roland Côté, Guy Charpentier

1Université du Québec à Trois-Rivières, 3551, boulevard des Forges, Trois-Rivières, Québec, G9A 5H7, Canada.

Address for correspondence: guy.charpentier@uqtr.ca

**PATHOGENICITY OF THRIPINEMA FUSCUM TIPPING & NGUYEN**

Kelly R. Sims, James J. Beecle, Joseph E. Funderburk, Drion G. Boucias

1Entomology and Nematology Department, University of Florida, Building 970 Natural Area Drive, PO Box 110620, Gainesville, FL 32611, USA.

2Center for Medical, Agricultural and Veterinary Entomology, USDA/ARS, 1600 SW 23rd Drive, Gainesville, FL 32608, USA.

3North Florida Research and Education Center, University of Florida, 155 Research Road, Quincy, FL 32351, USA.

Address for correspondence: simsk@ufl.edu

**POSTER / NEMATODE. WEDNESDAY, 16:30. N-02**

Habitat complexity effects on movement of *Steinernema carposcapae* in maize

Randa Jabbour, Mary E. Barbercheck

1Intercollege Graduate Degree Program in Ecology, The Pennsylvania State University, University Park, PA 16802, USA.

2Department of Entomology, The Pennsylvania State University, 501 ASI, University Park, PA 16802, USA.

Address for correspondence: meb34@psu.edu

**POSTER / NEMATODE. WEDNESDAY, 16:30. N-04 STU**

SUSCEPTIBILITY OF THE COLORADO POTATO BEETLE TO THE NEMATODE *Pristionchus uniformis*

Andreas M. Weller, Ralf J. Sommer

1Department for Evolutionary Biology, Max Planck Institute for Developmental Biology, Spemannstrasse 35-37, 72076 Tübingen, Germany.

Address for correspondence: andreas.weller@tuebingen.mpg.de
Cloning and expression of a venom protein from the endoparasitoid, *Pimpla hypochondriaca*, which has haemocyte anti-aggregation activity in vitro

M. P. Dani¹; E. H. Richards¹
¹Central Science Laboratory, Sand Hutton, York, Y041 1LZ, UK.
Address for correspondence: p.dani@csl.gov.uk

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-02**

A recombinant immunosuppressive protein from *Pimpla hypochondriaca* increases the susceptibility of two lepidopteran pests to *Bacillus thuringiensis*

E. H. Richards¹; M. P. Dani¹
¹Central Science Laboratory, Sand Hutton, York, Y041 1LZ, UK.
Address for correspondence: e.richards@csl.gov.uk

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

Yun-Shiang Chang¹; Wang-Jing Liu¹; Tsung-Lu Chou¹; Yuan-Ting Lee¹; Tai-Lin Lee¹; Wei-Tung Huang¹; Chu-Fang Lo²
¹Department of Molecular Biotechnology, Da-Yeh University, Changhua 515, Taiwan; ²Department of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario, N1G 2W1.
Address for correspondence: yscang@mail.dyu.edu.tw

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

Wang-Jing Liu¹; Yun-Shiang Chang¹; Hao-Ching Wang³; Jann-Hong Lee⁴; Guang-Hsiung Kou¹; Chu-Fang Lo²
¹Institute of Zoology, National Taiwan University, Taipei 106, Taiwan; ²Department of Molecular Biotechnology, Da-Yeh University, Changhua 515, Taiwan; ³Institute of Biochemical Sciences, National Taiwan University, Taipei 106, Taiwan.
Address for correspondence: d92b41004@ntu.edu.tw

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 ^32P-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 communoprecipitation 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 spheroedin promoter

Srinidhi C. Perea¹; Peter J. Kreil²; Basil M. Arif³
¹Great Lakes Forestry Centre, Canadian Forest Service, Laboratory for Molecular Virology, Sault Ste. Marie, Ontario, P6A 2E5, Canada; ²Department of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario, N1G 2W1, Canada.
Address for correspondence: aperea@nrcan.gc.ca

Spheroedin (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
Tze-Yong Jiun1; Tsung-Lu Chou1; Suey-Sheng Kao1
1Taiwan Agricultural Chemicals and Toxic Substances Research Institut, 11, Kuang-Ming Rd., Wufeng, Taichung Hsien, Taiwan, R.O.C.
Address for correspondence: jihn@tactri.gov.tw

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 thus the effects of chitinase J (Chi J) from Jelly Fig (Ficus ovateosang) 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 Trichophasia ni larvae were treated by the 1mg/ml of Chi J combined with 1x10^8 PBs/ml of AcMNPV. Results showed that the LD_{90} value was reduced from 1.46 x 10^8 PBs/ml to 1.1 x 10^7 PBs/ml. Further, we employed the internal ribosome entry site (IRES) element of rhopalosiphan padii virus (RhPV) to construct pAcPp6.9Chi-J-EGFP for Chi J and EGFP genes expression. We expect that the polyhedrin-positive recombinant baculovirus vAcPp6.9Chi-J-EGFP will enhance the insecticidal efficacy of baculovirus in infected Trichophasia ni larvae.

Poster / Virus. Wednesday, 16:30. V-05
Reprogramming expression of chitinase and cathepsin of the Autographa californica multiple nucleopolyhedrovirus
Jeffrey J. Hodgson 1; Noha Gerges 1; Peter J. Krell 1
1University of Guelph, Guelph, ON, N1G 2W1, Canada; Great Lakes Forestry Centre, Sault Ste. Marie, ON, P6A 2E5, Canada.
Address for correspondence: pkrell@uoguelph.ca

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
Marina Biedma1; Carolina Jaquenod De Giusti1; Alejandra Carrea1; Marcelo Beretta1; Alicia Sciocco-Cap2; Victor Romanowski1
1Universidad Nacional de La Plata, IBBM (UNLP-CONICET), Facultad de Ciencias Exactas, calle 49 y 115, (1900) La Plata, Argentina, 2Instituto Nacional de Tecnología Agropecuaria (INTA), IMYZA-CICVYa, CC 25 (1712) Castelar, Buenos Aires, Argentina.
Address for correspondence: victor@biol.unlp.edu.ar

Epinotia apurema (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 climates the insecticidal activity of both viruses needs improvement in order to compete with chemical pesticides. For this 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 infection 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 iel 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.
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 xyina multiple nucleopolyhedrovirus (LxyxMNPy) 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 LxyxMNPy 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 LxyxMNPy-infected SF21-CE 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 LxyxMNPy-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 LxyxMNPy-infected LD and SF cells.

Functional analysis of the putative antiapoptotic genes, p49 and iap4, of Spodoptera litura nucleopolyhedrovirus with RNAi

A homology search of public database revealed that Spodoptera litura nucleopolyhedrovirus (SpitNPV) 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 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 viabilities analysis showed that Splt-IAP4 has 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.

Cypoviruses, a member of the family Reoviridae, are one group of insect viruses 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 is located about 10Å along 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 of high resolution X-ray powder diffraction, the structural analysis of the polyhedrin is conducted by X-ray powder diffraction in SPring-8.
Identification of viral factors required for the enhancer-like function of baculovirus polyhedrin upstream (pu) sequence
Carol P. Wu1; Tou-Ya Huang1; Jen-Yeu Wang1; Hsueh-Hua Lo1; Yu-Chan Chao1
1Institute of Molecular Biology, Rm521, Academia Sinica, Nankang, Taipei115, Taiwan, R.O.C.
Address for correspondence: carolwu@imb.sinica.edu.tw

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 cosmids 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.

Identification of putative miRNA sequences in four insect pathogenic viruses
Woojin Kim1; John P. Burand1
1University of Massachusetts - Amherst, Fernald Hall, Amherst, MA 01003, USA.
Address for correspondence: jburand@microbio.umass.edu

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 -25 kcal/mol. These pre-miRNAs are then cleaved into a ~22 nt single stranded active miRNA. Here we report on our analysis of the genomes of four insect pathogenic, DNA viruses that 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 castaneum, (~9,000), Anopheles gambiae, (~8,500), 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.

MicroRNAs expressed in larval gypsy moth cells post parasitization by Glyptapanteles flavicoxis parasitoid
Dawn Gundersen-Rindal1;2 USDA, Invasive Insect Biocontrol and Behavior Laboratory, Biological Control, 211 Bldg 011A, Rm 214, BARC West, Beltsville, MD 20705, USA.
Address for correspondence: dawn.gundersen-rindal@ars.usda.gov

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/GBV-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.

A Metagenomics approach was used to identify unknown organisms which live in association with the gypsy-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 castaneum, (~9,000), Anopheles gambiae, (~8,500), 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.
Applying an *Anticarsia gemmatalis* multiple nucleopolyhedrovirus (AgMNPV)-based direct cloning system to make a DNA expression library of the cottonwood borer beetle (*Plectrodera scalator*).

Jeffrey M. Slack; Olga Lihoradova; Irina Ogay; Shakhnoz Azimova; John Dedes; Rian Schwarz; Peter J. Krell; Basil M. Ariń

1Great Lakes Forestry Centre, Sault STE Marie, ON P6A 2E5, Canada; 2Institute of Chemistry of Plant Substances, Tashkent, Uzbekistan; 3Department of Biochemistry, Cambridge University, Cambridge, CB2 1QW, UK; 4Department of Molecular and Cellular Biology, University of Guelph, Guelph, ON N1G 2W1, Canada.

Address for correspondence: js slack@nrcan.gc.ca

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 AgMNPV 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.

Optimization for high-throughput expression of recombinant protein using EasyBac system

Jae Young Cheol; Yang-so Kim; Hee Jin Shim; Yong Wang; Jong Yul Roh; Soo Dong Woo; Byung Rae Jin; Yeon Ho Je

1Research Institute for Agriculture and Life Sciences, Seoul National University, 599 Gwanakro, Gwanak-gu, Seoul 151-742, Korea; 2Department of Agricultural Biotechnology, Seoul National University, 599 Gwanakro, Gwanak-gu, Seoul 151-742, Korea; 3College of Agriculture, Life & Environments Sciences, Chungbuk National University, 410 SungBong-Ro, Heungdok-gu, Cheongju, Chungbuk 361-763, Korea; 4College of Natural Resources and Life Science, Dong-A University, 840 Hada2-dong, Saha-gu, Busan 604-714, Korea.

Address for correspondence: bturs@snu.ac.kr

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* braconvirus (CyBPV) 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
Chi-Hon Liao1; Yi-Ting Lin1; Tsong-Yuan Wu1

1Department of Bioscience Technology, Chung Yuan University, Chungli, Taiwan.
Address for correspondence: tywu@cycu.edu.tw

We had identified the RhPV IRES (derived from Rhopalisosiphum padii virus) and PnV539 IRES (derived from Perina nada 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 (OmpNPV). 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 baculovirus vectors 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
Fernanda Murgaia-Meca1; Richard B. Hitchman2; Linda A. King1
1Insect Virus Research Group, Oxford Brookes University, Headington Campus, Gipsy Lane, Oxford OX3 0BP, UK 2Oxford Expression Technologies Ltd., Oxford Brookes University, Headington Campus, Gipsy Lane, Oxford OX3 0BP, UK. Address for correspondence: fernanda.murgaia@brookes.ac.uk

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 GalNac-T3. 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
Namjune Kim1; Young-Moo Choo2; Kwang-Sik Lee2; Seong-Jin Hong1; Kwang-Youl Soo1; Byung-Rae Jmi

1Department of Agricultural Biology, NIAST, RDA, 61 Seodon-dong Gwonseon-gu, Suwon 441-853, Korea. 2Department of Applied Biology, College of Natural Resources and Life Science, Dong-A University, 840 Hadam2-dong, Saha-gu, Busan 604-714, Korea. Address for correspondence: vastni@rda.go.kr

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, is 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 cellulase 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
Olga A. Lihoradova1, Irina D. Ogay1,2, Maria M. Podpisnova1, Shakhnoz S. Azimova3,4 Institute of the Chemistry of Plant Substances, The Academy of Sciences of Uzbekistan, Mirzo-Ulugbek St., 77, Tashkent, Uzbekistan 3Department of Biochemistry, University of Cambridge, Sanger Bldg., Cambridge, CB2 1GA, UK. Address for correspondence: olga_lab@yahoo.com

Mullerian Inhibiting Substance (MIS) is a member of the transforming growth factor b (TGFβ) 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.

**Persistent infection and vertical transmission of Spodoptera exigua multiple nucleopolyhedrovirus (Hiibner) (Lepidoptera: Noctuidae)**

Oihana Cabodevilla,1 Oihane Simón,1 Delia Muñoz,1 Primitivo Caballero,2 Trevor Williams1

1Instituto de Agrobiotecnología, CSIC, Universidad Pública de Navarra, Gobierno de Navarra, 31192 Mutxila Baja, Navarra, Spain; 2Instituto de Ecología AC, Xalapa, Veracruz 91070, Mexico.

Address for correspondence: oihana.cabodevilla@unavarra.es

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.

**Hypermobility and climbing behaviour induced by baculovirus infection are regulated by separate gene functions**

Kelli Hoover1, Monique M. van Oers2

1Pennsylvania State University, 501 ASI Building, University Park, PA 16802, USA; 2Wageningen University, Binnenhaven 11, 6709 PD, Wageningen, The Netherlands.

Address for correspondence: kkh25@psu.edu

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.

**Comparative pathology of the slow-killing Adoxophyes honmamai NPV and Autographa californica MNPV in A. honmamai**

Daigo Fujita1; Takayoshi Ishii1; Yasushina Kunimi1; Madoka Nakai1

1Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan.

Address for correspondence: madoka@cc.tuat.ac.jp

Adoxophyes honmamai nucleopolyhedrovirus (AdhoNPV) has a distinctive slow-killing pathology compared to most other typical NPVs, such as *Autographa californica* MNPV (AcMNPV). Neonate *A. honmamai* 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. honmamai* 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 for AcMNPV. The gene encoding ecysteoid 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.

**Low oral infectivity of AcMNPV in Anticarsia gemmatalis larvae correlates with hemocyte resistance to infection by budded virus.**

Eric J. Haas-Stapleton; Maggie Carrera; Tiffany Chen; Aniska Chikhalya; Alisa de la Cruz; Marianne Torres

1Biological Sciences, California State University, Long Beach, 1250 Bellflower Blvd., Long Beach, CA 90840, USA.

Address for correspondence: ehas@csulb.edu

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 (AcMNPV), in *A. gemmatalis* larvae using an AcMNPV recombinant carrying the lacZ reporter gene (AcMNPV-hp70lacZ). 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-elicted 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 AcMNPV. Because neither AcMNPV OB nor BV were highly pathogenic in *A. gemmatalis* larvae, AcMNPV may not be effective in organic cropping systems for controlling *A. gemmatalis* larvae.
The Codling moth (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 2-Chromosome. A homogeneous resistant codling moth strain was generated by inbreeding a semi-resistant field strain. The LC₅₀ 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 CpGV-Bacmid expressing the green fluorescent protein (gfp) was constructed.

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 plasma from two strains of Cp (CpGV-susceptible (CpW and CpSv) and CpGV-resistant (CpRR1 and CpRGV)).

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.
Comparative sequence analysis of two entomopoxviruses (EPVs) from Choristoneura biennis and Choristoneura rosaceana

Zhen Li1; Christopher Lucarotti2; Peter J. Keel3; Basil M. Arti4
1Laboratory for Molecular Virology, Great Lakes Forestry Centre, 1219 Queen St. East, Sault Ste. Marie, Ontario, P6A 2E5, Canada, 2Atlantic Forestry Centre, 1350 Regent Street South, Fredericton, New Brunswick, E3B 5P7, Canada, 3Department of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario, N1G 2W1, Canada.

Address for correspondence: zhenli@nrcan.gc.ca

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 ornamentals. 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 both 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 betaentomopoxvirus. Phylogenetic analysis of spheroidin indicates that CBEV, CREV, CFEV and HAEV are very close to AMEV.

A new entomopoxvirus isolated from tea tortrix, Homona coffearia, in Sri Lanka

Kiri Asano1; Keerthi Mohotti2; Yasuhashi Kunimi1; Madoka Nakai1
1Tokyo University of Agriculture and Technology, Fuchu, Tokyo183-8509, Japan, 2Tea Research Institute of Sri Lanka, Talawakelle, Sri Lanka.

Address for correspondence: 50007534001@st.tuat.ac.jp

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. magninana in Japan, which was initially isolated from Adoxophyes honinai (AdhoEPV). We first compared the new Sri Lankan EPV and the Japanese AdhoEPV by analyzing their restriction endonuclease (REN) profiles and pathology in H. magninana. 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. magninana populations in Japan.

Determining the influence of transposon TCl4.7 insertion on the function of the genome of Cydia pomonella granulovirus

Menofy Wael H. El; Correal Carlos Espinel-Correal1; Xavier Léry1; Laura F. Villamizar1; Alba M. Cotes1; Miguel López-Ferber1; Cespinel Carlos Espinel-C
1Laboratory of Biotechnical Crop Protection, Dept.of Phytopathology, Agricultural Service Center Palatinate (DLR-Rheinfallz), Breitenweg 71, 67435 Neustadt/Weinstraß, Germany
2Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Center (ARC), 9 Gamaa Str., Giza, Egypt.

Address for correspondence: wael.elmenofy@dlr.rlp.de

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 Mccp5. 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 infected larvae. However, a significant decrease of Cp15 and Cp16 transcripts could be observed in Mccp5. 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 mimics. Both mutant Bacmids could 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.
The *Glossina pallidipes* salivary gland hypertrophy virus (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 GpsGHV 005 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

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, the stress protein, heat shock protein 70, and DWV viral load between symptomatic, asymptomatic, and uninfected adults. Large collections of entomopathogenic fungi are maintained 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.
Field performance of novel stacked Bt products for protection against corn insects

Ken Narva1, Mike Cuty1, Paul Neese1, Ed King1, Gary Thompson1
1Dow AgroSciences, 9330 Zionsville Road, Indianapolis, IN 46268, USA.
Address for correspondence: knarva@dow.com

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 Cry34/Ab1 and Cry35Ab1, event DAS-59122-7). The Cry1F component of HERCULEX® XTRA protects 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 Cry34/Ab1/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.

Development of and prospects for the BtBooster platform technology

Milton D. Taylor1, Mohd Amir F. Abdullah1, Laura N. Frame1; Michael J. Adang2
1 Insectigen, Inc., 425 River Road, Athens GA 30602 USA, 2 The University of Georgia, 427 Biological Sciences, 120 Cedar St., Athens GA 30602 USA.
Address for correspondence: taylor@insectigen.com

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.
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 biocide industry to provide stewardship for all products on the market.

Symposium. Thursday, 8:30. 181

Bacterial insecticides, commercial development and quality control
Changyan Chen

1Certis USA LLC, 9145 Guilford Road, Suite 175, Columbia, MD 21046, USA.
Address for correspondence: cychen@certisusa.com

The development of bacterial insecticides 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 insecticides. 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, (4) 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 measurement of the dose response 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 bioassy to determine potency. The limitations of using the alternative methods will be presented and discussed.

Symposium. Thursday, 8:00. 180

Bt standards and the importance of quality control of Bt products
Terry A. Benson

1Valent BioSciences Corporation, 6131 Oakwood Rd, Long Grove IL 60047, USA.
Address for correspondence: terry.benson@valent.com

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 microbial biocontrol agents - results of the REBECA Action
Ralf-Udo Ehlers

1University of Kiel, Germany.
Address for correspondence: ehlers@biotec.uni-kiel.de

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 quantities, 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 track 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.
The recovery of Mimivirus (for “Mimicking Microbe” virus) in 2001, a double-stranded DNA virus infecting common amoeba of the *Acinetobacter* 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 its particle size (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.

The genome of *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 other large DNA 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, vsf-1, and lef-5), replication (dnapol and helicase), virus structure (p74, pif-1, pif-2, pif-3, vp91, vp39, 39k, 19kda, and odf-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 *pifs* 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.
Apis mellifera. The most prevalent virus infecting honey bees in recent years, associated wasps.

Wasp-bracovirus associations: The grail quest for the ancestor virus

Annie Bézier1; Marc Annaheim1; Juline Herbinière1; Christoph Wetterwald1; Gabor Gyapay1; Sylvie Bernard-Samain1; Patrick Wincker1; Isabel Roditi2; Manfred Heller2; Maya Belghazi3; Jérôme Lesobre1; Rita Pfister-Wilhem1; Georges Periquet1; Catherine Dupuy1; Elisabeth Huguet1; Nathalie Volkoff2; Beatrice Lanzrein2; Jean-Michel Drezen1

1IRBI CNRS, University of Tours, Parc de Grandmont, 37200 Tours, France; 2Institute of Cell Biology, University of Bern, Switzerland; Balzertstrasse 4, CH-3012 Bern, Switzerland; 3Department of Clinical Research, University of Bern, CH-3012 Bern, Switzerland; Genoscope, Centre National de Séquençage, 2 rue Gaston Crémiex, CP5706 91057 Evry cedex, France. 4Proteomic analysis center, Faculté de Médecine Secteur Nord, 13916 Marseille, France; 5BIHV INRA, Université de Montpellier II, pl Eugène Bataillon, 34090 Montpellier, France.

Address for correspondence: drezen@univ-tours.fr

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. 49

A sticky situation: Picorna-like viruses infecting U.K. honeybee populations

Andrea C. Baker1; Aliya El Nagar1; Luke McKenzie1; Matt J. Hall1; Declan C. Schroeder1

1Marine Biological Association of the United Kingdom, Citadel Hill, Plymouth, PL2 2BP, UK.

Address for correspondence: ancba@mba.ac.uk

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, VDY 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.

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. 91

Honeybee immunity and parasitism by Nosema spp. fungi and Varroa mites

Catherine M. Little1; Dave Shuter2

1Acadia University, 24 University Avenue, Wolfville, NS B4P3R6 Canada.

Address for correspondence: 0764441@acadiau.ca

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.
Nosemosis in western honey bees (Apis mellifera) is caused by the microsporidian Nosema ceranae and N. apis. 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) was successful at temporarily reducing this recent invasive parasite in vitro. When compared to controls, fumagillin 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.

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.
Symposium. Thursday, 14:24. 196

Covert viruses in wild populations
Rosie S. Hailes*, 1 NERC Centre for Ecology and Hydrology, Oxford, UK. Address for correspondence: rha@ceh.ac.uk

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
Leellen F. Solter*, 1 Illinois Natural History Survey, Illinois, USA. Address for correspondence: lsolter@uiuc.edu

Entomopathogenic microsporida 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, SasaJschymnus tsgae, of the hemlock woolly adelgid, Adelges tsugae.

Symposium. Thursday, 15:12. 198

Methods for studying pathogens in natural populations: Recent developments and future thoughts
Helen Hesketh*, 1 NERC Centre for Ecology and Hydrology, Mansfield Road, Oxford, OX1 3SR, UK. Address for correspondence: hhesketh@ceh.ac.uk

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 philology as pathogens may occur at 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
Alison M. Dunn*, 1 Biological Sciences, University of Leeds, LS2 9JT, UK. Address for correspondence: a.dunn@leeds.ac.uk

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 nulleri 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 Echinorhynchus 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 Theholania 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.

Symposium. Thursday, 15:36. 200

Contributed papers Thursday, 14:00-15:30

BACTERIA 4

Genetic improvement of the Cry11 from Bacillus thuringiensis subsp. medellin by directed molecular evolution
Alvaro M. Florez1; Gloria M. Morales2; Sergio Onduz3
1Universidad de Santander, Laboratorio de Biología Molecular y Biotecnología, 3-201, Arahuanco Building, Calle 70 No. 55-210, Bucaramanga, Colombia, 2Universidad Nacional de Colombia sede Medellín y Corporación para Investigaciones Biológicas, Unidad de Biotecnología y Control Biológico, Carrera 72 A No 75B-141, Medellín, Colombia. Address for correspondence: amflorez@udes.edu.co

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. jugaeasens) 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
Qi Peng1,2; Li Zhu1; Fuping Song1; Lie Zhang1; Jiguao Gao2; Dafang Huang1
1State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193 China, 2College of Life Sciences, Northeast Agricultural University, Harbin 150030 China,
3Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing 100081 China.
Address for correspondence: songfp@hotmail.com

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 respectively. 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 gene 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
Miao M. Hang1; Liang Xiao1; Jun Cai1; Chi C. Xie1; Yuchu Chen1,2
1Department of Microbiology, College of Life Sciences, Nankai University, Weijin Road, Tianjin, 300071, P.R.China, 2Key Laboratory of Molecular Microbiology and Technology, Ministry of Education, Weijin Road, Tianjin, 300071, P.R.China. Address for correspondence: yychen@nankai.edu.cn

Bacillus thuringiensis strain 519-1 were tested for the antagonistic activity against the growth of hyphae of eight fungi including Aspergillus 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 cultur of 519-1 were tested for the antagonistic activity against the growth of hyphae of eight fungi including Aspergillus 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 cultur of 519-1 exhibited high toxicity against Helicoverpa armigera and Spodoptera exigua, with LC50 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, 15:00. 204

Pathogenesis of male-killing Wolbachia in Drosophila bifasciata
Aurore Dubuffett1, Zoe Veneti1, Henk R. Braig2, Judith E. Smith1, Greg D. D. Hurst1
1Faculty of Biological Sciences, University of Leeds, Leeds, UK, 2School of Biological Sciences, University of Liverpool, Liverpool, UK.
Address for correspondence: a.dubuffet@leeds.ac.uk

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, pathogenesis, 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.
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, M ascidifurax raptor, only slight effects were noticed. In addition, no trirophic 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
Sijun Liu1; Zhaohui Wang2; S. Sivakumar3; Liljana Georgievska1; Glenn F. King1; W. Allen Miller1; Bryony C. Bonning1
1Iowa State University, Department of Entomology, Ames, IA 50011, USA, 2Iowa State University, Department of Plant Pathology, Ames, IA 50011, USA, 3Institute for Molecular Bioscience, Brisbane, QLD 4072, Australia.
Address for correspondence: bbonning@iastate.edu

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 aphical 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.
Heterologous expression of recombinant bacterial endochitinases and production of chitin-derived oligosaccharides

J. Eleazar Barboza-Corona1; O. B. Gutierrez-Acosta1; M. Imperial-Cervantes1; Dennis K. Bideshi1,4; N. de la Fuente-Salcido1,2; R. Salcedo-Hernandez2

1Universidad de Guanajuato, Instituto de Ciencias Agrícolas, Departamento de Ingeniería en Alimentos, Irapuato, Guanajuato, Mexico, 36500, 1Universidad Autónoma de Coahuila, Escuela de Ciencias Biológicas, Torreón Coahuila, Mexico, 27440, 2California Baptist University, Department of Natural and Mathematical Sciences, 8432 Magnolia Avenue, Riverside, California 92504, USA, 3University of California, Riverside, Department of Entomology, Riverside, California, 92521, USA.

Address for correspondence: dbideshi@calbaptist.edu

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, chA NIMA from Serratia marcescens and chA74 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 heterologously synthesized in E. coli.

Contributed paper. Thursday, 14:45. 209

Use of a granulovirus (PoGV) and Bacillus thuringiensis (Bt) to control potato tuber moth (Phthorimaea operculella)

Steven P. Arthur1, Lawrence A. Lacey1, O. B. Gutierrez1, N. de la Fuente1, R. Salcedo2, M. Imperial1, Dennis K. Bideshi1,4, J. Eleazar Barboza-Corona1, Elizabeth W. Davidson1,4, Donald Lightner3,4, University of Arizona Agriculture Center, Maricopa, AZ, USA, 1Yakima Agricultural Research Laboratory, USDA-ARS, 5230 Konnowac Pass Rd, Wapato, WA, USA.

Address for correspondence: stevenarthurs@hotmail.com

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 90.3% 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, diamonaceous 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. Thursday, 15:15. 211

Finding a microbial control agent for the invasive crayfish, Orconectes virilis

Elizabeth W. Davidson1, Jennifer L. Snyder2, Donald Lightner3, 1University of Arizona Agriculture Center, Maricopa, AZ, USA, 1School of Life Sciences, Arizona State University, Tempe AZ 85287-4501, USA, 1University of Arizona Agriculture Center, Maricopa, AZ, USA, 1Veterinary Science/Microbiology, University of Arizona, Tucson, AZ, USA.

Address for correspondence: e.davidson@asu.edu

Arizona had 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.

Contributed paper. Thursday, 15:30. 213

Finding a microbial control agent for the invasive crayfish, Orconectes virilis

Elizabeth W. Davidson1, Jennifer L. Snyder2, Donald Lightner3, 1University of Arizona Agriculture Center, Maricopa, AZ, USA, 1School of Life Sciences, Arizona State University, Tempe AZ 85287-4501, USA, 1University of Arizona Agriculture Center, Maricopa, AZ, USA, 1Veterinary Science/Microbiology, University of Arizona, Tucson, AZ, USA.

Address for correspondence: e.davidson@asu.edu

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.

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.

Commercialisation of microbial control products: The industry perspective

**Wyn P. Grant**, 1, Justin G. Greaves 2; David Chandler 2; Gillian Davidson 2; G Mark Tatchell 3
1Department of Politics and International Studies, University of Warwick, Coventry CV4 7AL, UK; 2Warwick HRI, University of Warwick, Wellesbourne CV35 9EF, UK; 3Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, UK.
Address for correspondence: dave.chandler@warwick.ac.uk

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.

Understanding the adoption of alternative pest management strategies: An economist’s view

**Alastair Bailey**, Kent Business School, University of Kent, Canterbury, Kent CT2 7PE, UK.
Address for correspondence: alastair.bailey@imperial.ac.uk

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.
The Western corn rootworm (WCR) is one of the economically most important pests in corn. For its control, genes encoding *Bacillus thuringiensis* toxins (e.g., Cry3Bb1, Cry3A, Cry34Aa1/Cry35Ab1) were introduced into corn. The cultivation of transgenic corn expressing the respective *Bt*-toxins may result in the development of resistant pest populations. In general, the resistance of insects to *Bt*-toxins can be located at any step of the toxic pathway. However, in other *Bt*-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 (pNA) - 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 sarcosynolysin as well as the exopeptidases aminopeptidase and an omegapeptidase - acylaminocapeptidase were identified. For aspartic endopeptidases no specific pNA substrates were available. Using the general proteinase substrate azocaseine, the activity of the aspartic endopeptidase pepsin was demonstrated. Furthermore, with midgut epithelium binding analysis were carried out to study binding site competition of *Bt*-toxins Cry3Bb1 vs. Cry34Ab1/Cry35Ab1. From the midgut epithelium brush border membrane vesicles (BBMV) were prepared to examine the toxin binding. Biotin labeled *Bt*-corn-toxins, and the ligand-blot technique as well as streptavidin-horseradish-peroxidase-conjugat and the ECL system were used.

Contributed paper. Thursday, 16:30. 219

**B.t.-toxins in the midgut of Western corn rootworm** *(Diabrotica virgifera virgifera LeConte)*

Renate Kaiser-Alexnat, Federal Research Centre for Cultivated Plants, Julius Kuehn Institute (JKI), Institute for Biological Control, Heinrichstraße 243, D-64287 Darmstadt, Germany.

Address for correspondence: renette.kaiser-alexnat@jki.bund.de

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 binding 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, 17:00. 221

**Bacillus thuringiensis** Cry2A toxins bind saturably to a common site in the midgut of *Heliothis armigera*

C. Sara Hernández-Rodríguez1, Adri Van Vlier2, Nadine Bautsöens2, Jeroen Van Ric2, Juan Ferré1

1Universitat de València, Department of Genetics, Dr. Moliner 50, 46100-Burjassot (Valencia), Spain, 2Bayer BioScience N.V., Technologiepark 38, B-9052 Gent, Belgium.

Address for correspondence: juan.ferre@uv.es

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 amplification of antibiotic genes (*zwiterminic* 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 (*zwiterminic 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.
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 response to pathogen, 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 KDAs. 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.
“Here’s spitting at you, kid” - Oral transmission of the *Musca domestica* salivary gland hypertrophy virus (MdSGHV) via salivary secretions

Verena U. Lietze1; Christopher C. Gedeen2; Drion G. Boucias1
1University of Florida, Entomology and Nematology Department, 970 Natural Area Drive, Gainesville, FL 32611, USA. 2USDA-ARS, Center for Medical, Agricultural and Veterinary Entomology, 1600 SW 23rd Drive, Gainesville, FL 32608, USA.
Address for correspondence: vlietz@ufl.edu

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 103 to 106 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

Tamer Z. Salem1, 2; James E. Maruniak1; Verena U. Lietze1, Drion G. Boucias1
1Department of Entomology and Nematology, PO Box 110620, University of Florida, Gainesville, Florida 32611-0620, USA. 2Department of Microbial Molecular Biology, AGERI, Agricultural Research Center, Giza 12619, Egypt.
Address for correspondence: nzs2000@gmail.com

The *Musca domestica* salivary gland hypertrophy virus (MdSGHV) is a non-occluded, 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*, in Florida. The genome of MdSGHV has recently been sequenced (GenBank accession No.EU522111). The putative open reading frames (ORFs) showed similarity to *Glossina palpalis* 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 5′ terminus of MdSGHV transcripts (5′RACE) and/or the 3′ terminus (3′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, 16:30. 227

Isolation and functional analysis of an ascosivirus-encoded microRNA regulating viral replication

Mazhar Hussain1; Ryan J. Taft2; Sassan Asgari1
1School of Integrative Biology, University of Queensland, St Lucia QLD 4072, Australia. 2Institute for Molecular Bioscience, University of Queensland, St Lucia QLD 4072, Australia.
Address for correspondence: s.asgari@uq.edu.au

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 ascosivirus (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, 17:00. 229

Immobilization of proteins into Bombyx mori cypovirus polyhedra

Hajime Mori1; Hiroshi Iijii1; Gento Nishimura1; Takeshi Nakatanai; Keiko Ikeda1; Fasseli Coulibaly3; Elaine Chi1; Peter Metcalfe1
1Kyoto Institute of Technology, Kyoto, Japan. 2Protein Crystal Corporation, Osaka, Japan. 3University of Auckland, Auckland, New Zealand.
Address for correspondence: hmori@kit.ac.jp

Cypoviruses and baculoviruses are notoriously difficult to eradicate because the virus particles are embedded in micrometer-sized 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 three-dimensional 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 N-terminal domain of a cDNA fragment consisting of a compact three-layered sandwich core. We developed a new method for immobilization of foreign protein into the CPV polyhedra by the 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 BmCGP VP3.
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.

The impact of viruses on the aphid 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. 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 densivirus, 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.

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.
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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 41st Annual Meeting of the Society for Invertebrate Pathology and the 9th International Conference on *Bacillus thuringiensis* is gratefully acknowledged:

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Koppert Biological Systems
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