43rd ANNUAL MEETING OF THE SOCIETY FOR INVERTEBRATE PATHOLOGY

and

10 th International Colloquium on Invertebrate Pathology

The Final Meeting of COST862 Action: Bacterial Toxins for Insect Control

PROGRAM and ABSTRACTS

11-15 July 2010
Karadeniz Technical University,
Trabzon, Turkey

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PROGRAM
SIP 2010

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STU indicates papers being judged for graduate student presentation awards
136 indicates abstract number for ORAL presentation
N-05 indicates abstract number for POSTER presentation

ATTENTION:Photography of Scientific Presentations is not permitted.

Sunday July 11, 2010
Osman Turan Congress Center

07:30 Bus pick up at hotels

Senate Hall
8:00-17:00 **SIP Council Meeting**

Lunch and refreshments served

Sunday, 9:00-16:00
KTU UZEM, Department of Electrical Engineering

Bioinformatics Workshop
8000 Genomes at Your Fingertips: Comparative Genomics using CoGe
Dr. Eric Lyons, University of California, Berkeley, US

Lunch and refreshments served

Congress Centre
9:00-18:00 **Registration**

9:00-18:00 **Posters up**
Diseases of Beneficial Invertebrates, Microbial Control, Microsporidia, , Nematodes

18:30 Bus pick up at hotels

Congress Centre
19:00-21:30 Mixer

21:30 Buses return to hotels

Monday July 12, 2010
Osman Turan Congress Center

07:30 Bus pick up at hotels

Congress Centre
8:00-18:00 **Registration**

Monday, 8:30-10:00
Hasan Turan

Opening Ceremony and SIP Founders' Lecture

Opening Ceremonies
Prof. Dr. Zihni Demirbag,
Chair of Local Organizing Committee
Dr. Mark Goettel, President of the SIP
President of KTU
Students' Awards Ceremony

Founders' Lecture
Founder's Lecture, Introduction by Dr. James Becnel
Honoree: **Dr. Mauro Martignoni**
Lecturer: **Basil Arif**
Mauro Martignoni: A Renaissance Scientist in the 20th Century

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

10:00-10:30 **Posters Up**: Diseases of Beneficial Invertebrates, Microbial Control, Microsporidia, Nematodes

Plenary Symposium

Monday, 10:30-12:30
Hasan Turan

Biology of the Tsetse fly: Interactions with Parasites, Pathogens and Symbionts
Convener: Dr. Adly Abd-Alla, FAO/IAEA Agriculture and Biotechnology, Vienna, Austria.

10:30 **1** **Tsetse distribution and biology, and options and strategies for vector control**. Udo Feldmann, Insect Pest Control Section, Joint FAO/IAEA Division, Vienna, Austria.

11:00 **2** **Tsetse-transmitted trypanosomes - their biology and disease impact.** Peter Holmes, University of Glasgow Veterinary School, Scotland, GB.

11:30 **3** **Influences of the Symbiotic Fauna on Host Physiology**. Serap Aksoy, Yale University School of Public Health, New Haven, CT 06525, US.

12:00 **4** **Salivary Gland Hypertrophy Virus (SGHV): Impact on tsetse rearing and potential virus management strategies**. Adly Abd-Alla, Insect Pest Control Laboratory, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, Vienna, Austria

12:30-14:00 **LUNCH at KTU SAHIL**

Senate Hall
12:45-14:00 **ICTV Meeting**

Symposium Fungi

Monday, 14:00-16:00
Hasan Turan

Environmental Change and Entomopathogenic Fungi
Organizers: Helen Hesketh and Helen Roy

14:00 **5** **Influence of environmental temperature on insect-pathogen and insect-parasite interactions**. Matthew Thomas, Center for Infectious Disease Dynamics, Pennsylvania State University, US.

14:30 **6** **Modelling environmental gradients in host-pathogen systems**. ***Steven White, Rosie Hails.*** Centre for Ecology and Hydrology, Wallingford, GB.

15:00 **7** **Entomopathogenic fungi and invasional meltdown**. ***Helen Roy,*** Centre for Ecology and Hydrology, Oxfordshire, GB.

15:30 **8** **Life cycles of specialist insect pathogenic fungi: Can we expect any effects from environmental changes?** ***Jørgen Eilenberg***, Department of Agriculture and Ecology, Faculty of Life Sciences, University of Copenhagen, Thorvaldsensvej 40, DK 1871 Frederiksberg C, DK.

Contributed Papers

Monday, 14:00-16:00
Fahri Kuran

VIRUSES 1

Chairs: Hu Zhihong and Agah Ince

14:00 9 **Genome organization and expression of the translation products of providence virus: The type member of a new family of small insect RNA viruses?** Cheryl Walter[1], Fiona Pringle[2], Ritah Nakayinga[3], Pablo de Felipe[4], Martin Ryan[4], Andrew Ball[2] , Rosemary Dorrington[3], [1]Institute of Molecular and Cellular Biology, University of Leeds, Leeds, GB; [2]Dept. of Microbiology, University of Alabama at Birmingham, Birmingham, Alabama, US; [3]Department of Biochemistry, Microbiology and Biotechnology, Rhodes University, Grahamstown, ZA; [4]Centre for Biomolecular Sciences, University of St. Andrews, St Andrews, Scotland.

14:15 **10 Subcellular targeting of the *Helicoverpa armigera* stunt virus replicase: Evidence that tetraviruses replicate in association with membranes derived from the endocytic pathway**. James Short, Rosemary Dorrington, Department of Biochemistry, Microbiology and Biotechnology, Rhodes University, Grahamstown, ZA.

14:30 **11 Induction of an IAP antagonist in *Culex quinquefasciatus* larvae in response to infection by the baculovirus CuniNPV.** James Becnel[1], Liu Bo[2], Zhang Yanping[2], Zhou Lei[2], [1]Center for Medical, Agricultural and Veterinary Entomology, USDA/ARS, Gainesville, US; [2]Dept. of Molecular Genetics and Microbiology, Shands Cancer Center, University of Florida, Gainesville, US.

14:45 **12-STU The proteome of *Glossina pallidipes* Salivary Gland Hypertrophy Virus (*Hytrosaviridae*) virions**. Ikbal Agah Ince[1], Henry M. Kariithi[1], Sjef Boeren[2], Jacques Vervoort[2], Max Bergoin[3,] Just M. Vlak[1], Adly M. M. Abd-Alla[4], Monique M. van Oers[1], [1]Laboratory of Virology, Wageningen University, Droevendaalsesteeg 1, 6708 PB, Wageningen, NL; [2]Laboratory of Biochemistry,Wageningen University, Dreijenlaan , 6703 HA , Wageningen, NL; [3]Laboratoire de Pathologie Comparée, Université Montpellier-2, Place Eugène Bataillon, 34095 , Montpellier, FR; [4] Insect Pest Control Laboratory, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, A-1400 , Vienna, AT.

15:00 **13 Delineating the baculovirus secretome**. Ian Smith, Nara Institute of Science and Technology, Ikoma-shi, Nara, JP.

15:15 **14-STU Identification of an unusual structure found in Sf9 cells transfected with AcMNPV mutants defective in nucleocapsid assembly**. Ling Zhong, Meijin Yuan, Kai Yang, Yi Pang, State Key Lab of Biocontrol, Sun Yat-sen University, Guangzhou, CN.

15:30 **15 Histopathology of European decapod crustaceans exposed to White Spot Syndrome Virus (WSSV**). Kelly Bateman, Grant Stentiford, Cefas, Weymouth, GB.

15:45 **16 Gill-associated virus and protein subunit vaccination *in Penaeus monodon***. Darren Underwood, Jeff Cowley, Melony Sellars, Andy Barnes, Karyn Johnson, University of Queensland, St Lucia, AU.

Contributed Papers

Monday 14:00-16:00
Nihat Turan 1

BACTERIA 1

Chairs: Juan Luis Jurat-Fuentes and Hyun-Woo Park

14:00 **17 Comparison of virulence and virulence gene expression in *B. cereus* group bacteria during infection of *Galleria mellonella***. Kader Rebaine[1], Agnes Rejasse[1], Christophe Buisson[1], Eugenie Huillet[1], Claudia Bevilacqua[2], Christina Nielsen-LeRoux[1], [1] INRA Micalis1319, Guyancourt, FR; [2]INRA, Jouy en Josas, FR.

14 :15 **18-STU A novel approach for producing random mutation library with prospective base substitution rate for cry genes directed evolution**. Ming Liu[1], Changlong Shu[1], Chengxi Lin[1], Fuping Song[1], Dafang Huang[2], Jie Zhang[1], [1]State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, CN; [2]Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing, CN.

14:30 **19 Modification of nucleotide sequences between RBS and start codon in *Bacillus thuringiensis* resulted in increased level of cry2Ac mRNA**. Faiza Saleem[1,2], Hyun-Woo Park[2,3], Muhammad Akhtar[4], Abdul Rauf Shakoori[4], [1]School of biological sciences, PU ans Lahore College for Women University, [2]John A. Mulrennan, Sr., Public Health Entomology Research and Education Center Florida A and M University, Florida 32405, US; Lahore, PK; [3]California Baptist University, California [4]School of Biological Sciences, University of the Punjab, Lahore 54590, Pakistan, Lahore, PK.

14:45 **20 cDNAs and mRNA levels of aminopeptidase N protein genes from *Ostrinia furnacalis* strains with different susceptibilities to *Bt* toxins**. Lina Xu, Xueyan Chang, Kanglai He, Zhenying Wang, State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, CN.

15:00 **21 Proteomic characterization of the Cry1Ac-induced secretome in mature midgut cell cultures from *Heliothis virescens* larvae**. Anais Castagnola, Juan Luis Jurat-Fuentes, Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, US.

15:15 **22 Mutational analyses of loops in Domain II of *Bacillus thuringiensis* mosquitocidal Cry4Aa toxin.** Tohru Hayakawa, Yusuke Ono, Mohammad Howlader, Hiroshi Sakai, Okayama University, Okayama, JP.

15:30 **23 Description of the "ping pong" binding mechanism of Bt Cry1Ab toxin with *Manduca sexta* receptors**. Sabino Pacheco, Ivan Arenas, Isabel Gómez, Alejandra Bravo, Mario Soberón, Instituto de Biotecnología, Universidad Nacional Autónoma de México, Cuernavaca, Morelos, MX.

15:45 **24 Cyt1Aa synergizes the larvicidal activity of Cry2Aa against *Culex quinquefasciatus* through direct intermolecular interaction**. Dennis Bideshi[1], Greer Waldrop[2], Margaret Wirth[1], Jeffrey Johnson[1], Mercedes Diaz-Mendoza[1], Hyun-Woo Park[3], Brian Federici[4], [1]Department of Entomology, University of California, Riverside and Department of Natural and Mathematical Sciences, California Baptist University, Riverside, US; [2]Department of Biology

and Undergraduate Program in Molecular, Cellular, and Developmental Biology, University of Louisville, Louisville, US; [3]John A. Mulrennan, Sr., Public Health Entomology Research and Education Center, Florida AandM University and Department of Natural and Mathematical Sciences, California Baptist University , Riverside, US; [4]Department of Entomology and Interdepartmental Graduate Programs in Genetics and Cell, Molecular and Developmental Biology, University of California, Riverside, Riverside, US.

14:00-16:00 **View Posters**
Diseases of Beneficial Invertebrates, Microbial Control, Microsporidia, Nematodes (Authors stand by posters).

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

Symposium

Monday, 16:30-18:30
Hasan Turan

Biological Control of the Corn Rootworm (Diabrotica)

Organizer: Ken Narva

16:30 **25 The western corn rootworm in Europe: Current status and future challenges.** Stefan Vidal, Department of Crop Sciences Agricultural Entomology, Unversity of Goetingen, Goettingen, DE.

17:00 **26 Control of western corn rootworms with the entomopathogenic nematode *Heterorhabditis bacteriophora***. Ralf-Udo Ehlers, Kiel University, DE

17:30 **27 Challenges to resistance management for transgenic maize targeting the western corn rootworm**. Blair Siegfried[1], Timothy Nowatzki[2], Analiza Alves[1], Murugesan Rangasamy[1], [1]University of Nebraska, Lincoln, NE, US; [2]Pioneer Hi-Bred International, Johnston, IA, US.

18:00 **28 Assessing the impact of corn rootworm-resistant *Bt* maize on non-target predatory arthropods in laboratory and field studies**. Stefan Rauschen[1], Michael Meissle[2], Jörg Romeis[2], [1]RWTH Aachen University, Department of Plant Physiology, Aachen, DE; [2]Agroscope Reckenholz-Tänikon Research Station ART, Zurich, CH.

Contributed Papers

Monday, 16:30-18:30
Fahri Kuran

VIRUSES 2

Chairs: David Theilmann and Remziye Nalcacioglu

16:30 **29 The *Autographa californica* multiple nucleopolyhedrovirus occlusion-derived virus envelope protein ODV-E56 is required for oral infectivity but is not essential for virus binding and fusion**. Wendy Sparks[1], Bryony Bonning[1], Robert Harrison[2], [1]Department of Entomology, Iowa State University, Ames, Iowa, US; [2]Invasive Insect Behavior and Biocontrol Laboratory, Plant Sciences Institute, ARS, USDA, Beltsville, Maryland, US.

16:45 **30-STU Baculovirus *per os* infectivity factors form a complex on the surface of occlusion derived virus**. Ke Peng, Monique M. van Oers, Just M. Vlak, Jan W.M. van Lent, Lab of Virology, Wageningen University, Wageningen, NL.

17:00 **31 A peptide that binds the gut epithelium of *Heliothis virescens* has similarity to ODV-E66 and impedes infection with wild type baculovirus**. Wendy Sparks, Amy Rohlfing, Bryony Bonning, Iowa State University, Ames, US.

17:15 **32 Extensive proteomics analyses of the occlusion-derived virion of *Helicoverpa armigera* Nucleopolyhedrovirus**. Fei Deng[1], Ranran Wang[1], Dianhai Hou[1], Leike Zhang[2], Lin Guo[2], Hualin Wang[1], Zhihong Hu[1], [1]State Key Laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, CN; [2]Wuhan University and College of Life Sciences, Wuhan, CN.

17:30 **33 Proteomic analysis of the occlusion-derived *Amsacta moorei* Entomopoxvirus.** Srini Perera[1], Zhen Li[1], Min-Ju Chang[1], Hu Zhihong[2,] Remziye Nalçacıoglu[3], Zihni Demirbag[3], Peter Krell[4] and Basil Arif[1], [1] Laboratory for Molecular Virology, GLFC, Sault Ste Marie, ON, CA; [2] Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, CN;[3] Dept of Biology, Karadeniz Technical University, Trabzon, TR; [4]Molecular and Cellular Biology, University of Guelph, Guelph, ON, CA.

17:45 **34 Deletion and functionally analysis of Group I Alphabaculovirus ODV specific genes and replacement with Group II homologues**. Yingchao Nie[1], Minggang Fang[1], Christina McCarthy[1], Leslie Willis[1], Martin Erlandson[2], David Theilmann[1], [1]Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Summerland, CA; [2]Saskatoon Research Centre, Agriculture and Agri-Food Canada, Summerland, CA.

18:00 **35 The putative pocket protein binding site of *Autographa californica* nucleopolyhedrovirus BV/ODV-C42 is required for virus-induced nuclear actin polymerization**. Kun Li[1], Yun Wang[1], Huimin Bai1, Qian Wang[2], Jianhua Song[3], Yuan Zhou[1], Xinwen Chen[1], [1]State Key Lab of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, CN; [2]Department of Biochemistry and Molecular Biology, Nanjing Medical University, Nanjing, China, Nanjing, CN; [3]Cell Cycle and Cancer Biology Research Program, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma, Oklahoma City, US.

18:15 **36 *Autographa californica* multiple nucleopolyhedrovirus Ac92 (ORF92, P33) is required for budded virus production and multiple-envelope-occlusion-derived virus formation**. Wenbi Wu, Lorena Passarelli, Molecular, Cellular and Developmental Biology Program, Division of Biology, Kansas State University, Manhattan, Kansas 66502, US.

Contributed Papers

Monday, 16:30-18:30
Nihat Turan 1

BACTERIA 2

Chairs: Juan Ferre and Sabahat K. Ozman Sullivan

16:30 **37 Comparison of midgut proteolytic activity in susceptible and induced-resistant populations of *Plutella xylostella* to *Bacillus thuringiensis* subsp. *kurstaki* 3a3b**. Raziyeh Bakhshaei, Reza Talaei-Hassanloui, Vahid Hosseini-Naveh, College

of Agriculture and Natural Resources, University of Tehran, Karaj, IR.

16:45 **38 Molecular characterization of *Bacillus thuringiensis* using REP-PCR (Repetitive Element Polymorphism**). Rosane Silva-Fagundes[1], Edgard Picoli [2], Ubiraci Lana[1], Fernando Valicente[1], [1]Embrapa Maize and Sorghum Research Center, Sete Lagoas, BR; [2]UFV-Federal University of Viçosa, Viçosa, BR.

17:00 **39 Inheritance of Cry1Ac resistance in cotton bollworm, *Helicoverpa armigera* Hubner, and its implications in resistance management in Bt cotton**. G. T. Gujar, R Nair, V. Kalia, Division of Entomology Indian Agricultural Research Institute, New Delhi, IN.

17:15 **40 Characterization of binary toxin from *Bacillus sphaericus* ISPC-8**. Ramesh Hire[1], Ashok Hadapad[1], Vinay Kumar[2] , Tanaji Dongre[1], [1]Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Mumbai, IN; [2]High Pressure and Synchrotron Radiation Physics Division, Bhabha Atomic Research Centre, Mumbai, IN.

17:30 **41 Characterization of plasmid patterns of *Bacillus thuringiensis* efficient against *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae)**. Rosane Silva-Fagundes[1], Edgard Picoli [2], Ubiraci Lana[1], Fernando Valicente[1], [1]Embrapa Maize and Sorghum Research Center, Sete Lagoas, BR; [2]UFV-Federal University of Viçosa, Viçosa, BR.

17:45 **42-STU A new sugar phosphate sensor system in *B. cereus* induced during oral infection of *Galleria mellonella***. Qi Peng [1,2], Fuping Song[1,2], Julien Brillard[3], C. Buisson[4], J. Zhang[2], Dafang Huang[5], M de Been[6], T. Abee[6], V. Broussolle[3], D. Lereclus[1], C.Nielsen-LeRoux[1], [1]INRA, Micalis, Génétique Microbienne et Environnement, France. [2] State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, CAAS, China, Beijing, CN; [3]UMR408 INRA, Avignon, France, Avignon, FR; [4]INRA, Micalis, Génétique Microbienne et Environnement, FR; [5]Biotechnology Research Institute, CAAS, CN; [6]TI Food and Nutrition (TIFN) and Laboratory of Food Microbiology, Wageningen, NL

18:00 **43 Molecular characterization and insecticidal activity of a highly pathogenic isolate of *Bacillus thuringiensis* from Adana**. Semih Yilmaz[1], Abdurrahman Ayvaz[2], Mikail Akbulut[2], Ugur Azizoglu[1], Salih Karaborklu[3], Huseyin Basim[4], [1]Erciyes University, Graduate School of Natural and Applied Sciences, Kayseri, TR; [2]Erciyes University, Faculty of Sciences, Kayseri, TR; [3]Osmaniye Korkutata University, Faculty of Arts and Science, Osmaniye, TR; [4]Akdeniz University, Faculty of Agriculture, Department of Plant Protection, Antalya, TR.

18:15 **44 Cheap genomes: Identification and classification of spontaneous mutations by genome sequencing**. Eric Lyons, Sydney Kustu, William Inwood, Department of Plant and Microbial Biology, University of California, Berkeley, US.

Monday, 18:30-20:30
KTU SAHIL

DINNER

Monday 20:30-22:30

SIP DIVISION BUSINESS MEETINGS AND WORKSHOPS

Fungi Nihat Turan 1
Nematode Nihat Turan 2
Bacteria Hasan Turan

Virus Fahri Kuran

Business Meeting Workshop:Bioinformatics

Organizer: Eric Haas-Stapleton
The dynamic structure and evolution of genomes.
Eric Lyons, UC Berkeley, US

Microsporidia Preparation room

Business Meeting

Workshop: Genomics of microsporidia
Small, beautiful and sexy? Insights from a decade of microsporidian genomics.
Joe Ironside, Aberysytwyth University, Aberystwyth, GB

22:45 Buses return to hotels

POSTERS
Diseases of Beneficial Invertebrates, Microbial Control, Microsporidia, Nematodes

DİSEASES OF BENEFİCAL INVERTEBRATES

DBI-01 Demonstrating effective RNAi products to manage factors of honey bee colony collapse. Gal Yarden[1], Eitan Glick[1], Eyal Maori[2], Ilan Sela[2], Wayne Hunter[3], Jay Evans[4], Nitzan Paldi[1], Eyal Ben-Chanoch[5], [1]Beeologics, Inc, Rehovot, IL; [2]HUJI, Rehovot, IL; [3] USDA-ARS, Fort Pierce, US; [4]USDA-ARS, Beltsville, US; [5]Beeologics, Miami, Florida US.

DBI-02 Multiplex PCR detection of slowly-evolving trypanosomatids and neogregarines in bumblebees using broad-range primers. Ivan Meeus[1], Dirk deGraaf[1], Kris Jans[2], Guy Smagge[1], [1]UGent Lab Agrozoology , Gent, BE; [2]Biobest, Gent, BE.

DBI-03 Recent research on *Galleria mellonella* as one from most important insect model organisms. Ana Gabriela Osuna Paez[1], Pavel Hyrsl[2], [1]Consejo Estatal de Ciencia y Tecnología, Aguilar Barraza 1329 Centro Culiacán , Sinaloa, MX, [2]Deparment of Animal Physiology. Faculty of Science, Masaryk University, Brno, CZ.

DBI-04 Report of coccinellid and orius species of corn fields in Isfahan region. Jalalizand Ali Reza, Karimy Azadeh, Rezaii Nastaran. Isfahan, IR.

DBI-05 Determining microfungus flora of body surface and intestinal system of Caucasian race bees (*Apis mellifera caucasica* Pollmann, 1889) (Hymenoptera: Apidae). Mehmet Ali Kırpık[1], Mehmet Nuri Aydogan[2], Serkan Örtucu[2], İsmet Hasenekoglu[2], [1]Kafkas University, Kars, TR; [2] Ataturk University, Erzurum, TR.

MICROBIAL CONTROL

MC-01 Reduction of pesticides pollution by microbial control of soil-borne pests: A case study. Ali Derakhshan, Shahrood University of Technology, Shahrood, IR.

MC-02 Perspectives for determining bioactivity of three commercial lepidopteran microbial insecticides based on *Bacillus thuringiensis*. Ayyappan Nair[1], Terry Benson[1], Daniel Heiman[1], SenSeong Ng[2], John Isaacson[2], [1]Valent BioSciences Corporation, Long Grove, [2] Agriculture Testing Lab, Abbott Laboratories, North Chicago, US.

MC-03 The physiological characterization and functions of Poly-β-Hydroxybutyrate (PHB) in *Bacillus thuringiensis*. Chen Deju, He Jin, Zhang Qingye, Li Mingshun, Jiang Heng, Sun Ming, Yu Ziniu, Huazhong Agricultural University, Wuhan 430070, Hubei, P.R., CN.

MC-04 Monitoring on the resistance of diamondback moth to *Bacillus thuringiensis* (*Bt*) engineering strain WG-001. Congchong Zhang,[1] Yizhe Wang[1], Zhe Zhang[1], Jianglin Yao[1], Ziniu Yu[2], Jianhong Li[1], [1]College of Plant Science and Technology, Wuhan, 430070, CN; [2]State Key Laboratory of Agricultural Microbiology, National Engineering Research Center for Microbial Pesticides, Huazhong Agricultural University, Wuhan, 430070, CN.

MC-05 Characterization of culturable bacteria from *Ostrinia nubilalis* (Lepidoptera: Crambidae) and their insecticidal effects on the pest. Emrah S. Secil, Zihni Demirbag, Ismail Demir, Karadeniz Technical University, Trabzon, TR.

MC-06 Native isolates of fungal pathogens to control aphids in Uruguay. F. Rivas Franco, N. Altier, Rosario Alzugaray, INIA National Instute for Agricultural Research, INIA, UY.

MC-07 Microbiological control of the red palm weevil *Rhynchophorus ferrugineus* with *Beauveria bassiana* and *Metarhizium anisopliae*. Gian Paolo Barzanti, Pietro Rumine, Claudia Benvenuti, Valeria Francardi, C.R.A. – ABP Centro di Ricerca perl'Agrobiologia e la Pedologia , Firenze, IT.

MC-08 Determination of cellular immunity of insect pests collected from Kahramanmaras province, Turkey due to natural microbial infections. Hasan Tunaz[1], David Stanley[2], [1]KSU, Faculty of Agriculture, Department of Plant Production, Kahramanmaras, TR; [2]USDA/Agricultural Research Service, Biological Control of Insects Research Laboratory, 1503 S. Providence Road , Columbia, MO , US.

MC-09 Entomopathogenic fungi for locust control in the Republic of Georgia, a multifaceted evaluation. Jaronski Stefan[1], Abashidze Eleanora[2], Latchininsky Alexandre[3], Horowitz Rami[4], Aduashvili Gvanca[5], [1]USDA ARS, Sidney, US; [2]St. Andrew Georgian University, Tbilisi, GE.

MC-10 Initiation, characterization and karyotyping of a new cell line from red palm weevil *Rhynchophorus ferrugineus* adapted at 27°C. Khamiss Omaima, Abdel Badeea, Ahmed, El halfawy, Khaleel, GEBRI - MNF University, CAIRO, EG

MC-11 Protection afforded by an emulsifiable oil against imbibitional damage in *Metarhizium anisopliae* conidia. Marcos Faria[1], Rogerio Lopes[1], Solange Xavier-Santos[2], [1]EMBRAPA, Brasilia, BR; [2]Universidade Estadual de Goias, Anapolis, BR.

MC-12 The effects of host-plant resistance on the susceptibility of the diamondback moth to *Bacillus thuringiensis*. Maryam Jafary[1], Javad Karimzadeh[2], Mohammadreza Rezapanah[3], [1]Islamic Azad University, Arak, IR; [2]Department of Plant Protection, Isfahan Research Center for Agriculture and Natural Resources, Isfahan, IR; [3]Department of Biological Control, Iranian Research Institute of Plant Protectio, Tehran, IR.

MC-13 Entomopathogenic fungi sprayed at different times on *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs and parasitism by *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae). Michele Potrich[1], Pedro Neves[2], Luis Alves[3], Everton Silva[1], Jéssica Cavalcanti[3], Flavio Cechim[1], Ana Mamprim[3], Vanda Pietrowski[4], [1]Universidade Tecnológica Federal do Paraná , Dois Vizinhos, BR; [2]Universidade Estadual de Londrina, Londrina, BR; [3]Universidade Estadual do Oeste do Paraná, Cascavel, BR; [4]Universidade Estadual do Oeste do Paraná, Marechal Cândico Rondon, BR.

MC-14 Repellence of entomopathogenic fungi applied on *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs to the parasitism by *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae). Michele Potrich[1], Pedro Neves[2], Luis Alves[3], Everton Silva[1], Jéssica Cavalcanti[3], Flávio Cechim[1], Nicole Holz[3], Vandam Piettrowski[4], [1]Universidade Tecnológica Federal do Paraná, Dois Vizinhos, BR; [2]Universidade Estadual de Londrina , Londrina, BR; [3]Universidade Estadual do Oeste do Paraná, Cascavel, BR; [4]Universidade Estadual do Oeste do Paraná, Marechal Cândido Rondon, BR.

MC-15 Spread dynamics of *Hyphantria cunea* Drury in Georgia and its natural enemies. Nana Goginashvili[1], Meri Tvaradze[1], Manana Kereselidze[1], Archil Supatashvili[2], [1]Vasil Gulisashvili Forest Institute, Tbilisi, GE; [2]Tbilisi, GE.

MC-16 Appearance of pathogens within outbreak populations of native insect populations in New Zealand. Richard Townsend[1], Sean Marshall[1], Andreas Leclerque[2], Regina Kleespies[2], Tracey Nelson[1], Trevor Jackson[1], [1]AgResearch, Lincoln, NZ; [2]Julius Kuhn-Institut, Darmstadt, DE.

MC-17 The genome-scale metabolic network reconstruct and comparative analysis for *Bacillus thuringiensis* strain YBT-1520. Zhang Qingye, Yu Chan, Wang Yian, Xu Chenchen, He Jin, Ke Yun, Liu Ziduo, Sun Ming, Yu Ziniu, State Key Laboratory of Agricultural Microbiology, National Engineering Research Center for Microbial Pesticides, Huazhong Agricultural University, Wuhan430070, Hubei, P.R. CN.

MC-18 STU Investigation of the mode of action of toxins of *Bacillus thuringiensis* Cry1Aa and Cry1Ac and study of their interactions with the intestinal receptors of *Ephestia kuehniella.* Maissa Chakroun, Souad Rouis, Slim Tounsi, Samir Jaoua, Center of Biotechnology of Sfax, Sfax, TN.

MC-19 STU Microbial control of diamondback moth, *Plutella xylostella,* using entomopathogenic fungus *Beauveria bassiana* and *Lecanicillium* spp. Nahoko Nakagawa[1], Keika Yamada[2], Daigo Aiuchi[3], Toshio Masuda[4], Masanori Koike[1], [1]Department of Agro-envioromental Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, JP; [2]Graduate School

of Agriculture, Hokkaido University, Sapporo, Hokkaido, JP; [3]National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, JP [4]Miyagi prefecture Horicultural Experiment Station, Takadate, Natori, Miyagi, JP.

MICROSPORIDIA

M-01 **Effect of *Vairimorpha ephestiae* (Microsporidia: Burenellidae) on detoxifying and antioxidant enzymes in *Galleria mellonella* larvae**. Yana Vorontsova, Institute of Systematic and Ecology of Animals, Novosibirsk, RU.

M-02 **Intestine microsporidia of the genus *Liebermannia* from Argentine grasshoppers: Morphology, taxonomy, life cycles and routes for speciation.** Yuliya Sokolova[1], Carlos Lange[2], James Fuxa[3], [1]Institute of Cytology Russian Academy of Sciences, St.Petersburg, RU; [2]Louisiana State University AgCenter, Baton Rouge, LA, US.

M-03 **Subtraction analysis of *Nosema bombycis* infected IPLB-LD-652Y cell line.** Yu-Shin Nai[1], Chih-Yuan Wang[2], Tai-Chuan Wang[2], Chung-Hsiung Wang[2], Chu-Fang Lo[2], [1]Institute of Zoology, National Taiwan University, Taipei, TW; - [2]National Taiwan University, Taipei, TW.

M-04 **Effects of an ant species, *Formica fusca*, on the transmission of microsporidia infecting gypsy moth larvae.** Dorte Goertz, Gernot Hoch, University of Natural Resources and Applied Life Sciences Vienna, Boku, Vienna, AT.

M-05 ***Hepatospora eriocheiri* n.gn. n. sp. infecting Chinese mitten crabs (*Eriocheir sinensis*) and a proposal for erection of a new family (Basosporidae) to contain phylogenetically similar microsporidians from aquatic crustaceans.** Grant Stentiford[1], Kelly Bateman[1], James Munro[1], Aurore Dubuffett[2], David Stone[1], [1]Cefas, Weymouth, GB; [2]University of Leeds, Leeds, GB.

M-06 **Occurrence of *Nosema oryzaephili* in *Cryptolestes ferrugineus* and transfer to the genus *Paranosema*.** Jeff Lord[1], Charles Vossbrinck[2], Jeff Wilson[1], [1]USDA-ARS, Manhattan, Kansas, US; [2]Connecticut Agricultural Experiment Station, New Haven, Conn., US.

M-07 **Light microscopic and molecular detection of microsporidia infecting *Loxostege sticticalis* (Lepidoptera: Pyraustidae) in Eurasia.** Julia Malysh, Yuri Tokarev, Andrei Frolov, All-Russian Institute of Plant Protection, St. Petersburg-Pushkin, RU.

M-08 **Morphological and molecular variability in the *Nosema-Vairimorpha* species complex infecting *Lymantria dispar*.** Leellen Solter[1], Daniela Pilarska[2], Wei-Fone Huang[1], Philip Solter[3], Dorte Goertz[4], Gernot Hoch[4], Andreas Linde[5], Jiri Vavra[6], [1]Illinois Natural History Survey/University of Illinois, Urbana, Illinois, US; [2] Bulgarian Academy of Sciences, Sofia, BG; [3]University of Illinois, Urbana, Illinois, US; [4]BOKU–Universität fur Bodenkultur, Vienna, AT; [5]Fachhochschule Eberswalde, Eberswalde, DE; [6]University of South Bohemia, Branisovska, CZ.

M-09 **Transmission of a microsporidium from the convergent lady beetle, *Hippodamia convergens*, to the green lacewing, *Chrysoperla carnea* Stephens.** Samantha Curry, Susan Bjornson, Saint Mary's University, Halifax, CA.

M-10 **Fire ant microsporidia acquired by parasitoid flies of fire ants.** David Oi, Sanford Porter, Steven Valles, USDA-ARS Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, Florida, US.

NEMATODES

N-01 **STU Molecular cloning, expression and characterization of a serpine released by the entomopathogenic nematode *Steinernema carpocapsae*.** Monica Martinez Avila[1], Duarte Toubarro[1], N. Balasubramanian[1], Rafael Montiel[2], Y Jing1, Y-J. Hao[3], Nelson Simões[1], [1]CIRN and D. de Biologia, U. dos Açores. Apartado 1422. 9501-801 , Ponta Delgada, PT; [2]Laboratorio Nacional de Genómica para la Biodiversidad, CINVESTAV-IPN, Km 9.6 Libramiento Norte, Carretera Irapuato - León, CP 36821 Irapuato , Guanajuato, MX; [3]Biophysics, University of Chicago, 60637, Chicago, US.

N-02 **Clotting factors and eicosanoids protect against nematode infections**. Pavel Hyrsl[1], Pavel Dobes[1], Zhi Wang[2], Ulrich Theopold[2], [1]Depart. of Animal Physiology and Immunology, Institute of Experimental Biology, Masaryk University, 61137 Brno, CZ; [2] Depart. of Molecular Biology and Functional Genomics, University of Stockholm, 10691 Stockholm, SE.

N-03 **Innate immune responses of *Leptinotarsa decemlineata* and *Galleria mellonella* to the entomopathogenic nematodes, *Steinernema feltiae* and *Heterorhabditis bacteriophora* collected in Iran.** Laleh Ebrahimi[1], Gholamreza Niknam[1], Gary Brian Dunphy[2], [1]University of Tabriz, Iran, Tabriz, IR; [2]Department of Natural Resource Sciences, McGill University, Macdonald Campus, Quebec, CA.

N-04 **Symbiotic bacteria can modify the competitive success of sympatric *Heterorhabditis megidis* and *H. downesi* populations**. Tamas Lakatos, Timea Toth, Nemaform Ltd., Nagykallo, HU.

N-05 **A survey study on entomopathogenic nematodes in East Black Sea region of Turkey.** Cihan Gokce[1], Huseyin Yilmaz[2], Ismail Demir[1], Zihni Demirbag[1], [1]Karadeniz Technical University, Trabzon, TR; [2]Giresun University, Giresun, TR

N-06 **Effect of essential oils on entomopathogenic nematodes**. Dong Woon Lee[1], Young Hak Jung[2], Shin Hae Lee[3], Chung Gyoo Park[2], Sang Myoung Lee[3], Ho Yul Choo[2], [1]Kyungpook National University, Sangju, KR; [2]Gyeongsang National University, Jinju, KR; [3]Southern Forest Research Center, Jinju, KR.

N-07 **Parasitism of *Eurygaster integriceps* Puton (Heteroptera: *Scutelleridae*) by *Agamermis* sp. (Nematoda: Mermithidae)**. Gulcan Tarla[1], Sener Tarla[1], Mahmut Islamoglu[2], Gurhan Gun[3]. [1]Usak University, Vocational High School, Depart. of Organic Agriculture Program, 64800 Sivaslı, Usak, TR; [2]Plant Protection Research Institute, P.O. Box 21, 01321, Yuregir, Adana, TR; [3]Department of Plant Protection, Mustafa Kemal University, 03100 Antakya, Hatay, TR.

N-08 **Laboratory screening of the pathogenicity of some local entomopathogenic nematode isolates**

against the European cockchafer, *Melolontha melolontha* (Coleoptera: Scarabaeidae).
Huseyin Yilmaz[1], Cihan Gokce[2], Ismail Demir[2], Zihni Demirbag[2], [1]Giresun University, Giresun, TR; [2]Karadeniz Technical University, Trabzon, TR.

N-09 **Control potential of *Heterorhabditis bacteriophora* against a new turf pest, *Dorcadion pseudopreissi* (Coleoptera: Cerambycidae) in turf.** I. Alper Susurluk[1], N. Alper Kumral[1], Ugur Bilgili[2], Esvet Acikgoz[2]. [1]Uludag University, Agriculture Faculty, Plant Protection Depart., Bursa, TR; [2]Uludag University, Agriculture Faculty, Field Crops Depart., Bursa, TR.

N-10 **Biological control potential of an entomopathogenic preperation of *Heterorhabditis bacteriophora* on the white grub, *Polyphylla adspersa*.** Javad Karimi[1], Mohammad Reza Rezapanah[2], Fatemeh Monfared[1], Hossein Mirsaeidi[1], [1]Depart. of Plant Protection, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, IR; [2] Insect Virology Lab., Biocontrol Depart., Iranian Research Institute of Plant Protection, Tehran 19395, Iran, Tehran, IR.

N-11 **The effect of silver nanoparticles on the mortality of entomopathogenic nematodes from bioprepations.** Kornelia Kucharska, Dorota Tumialis, Elżbieta Pezowicz, Chair of Animal's Environment, Warsaw University of Life Sciences, Warsaw, PL.

N-12 **Sublethal impacts of Iranian isolates of *S. feltiae* and *H. bacteriophora* on the Colorado potato beetle, *Leptinotarsa decemlineata*.** Laleh Ebrahimi[1], Gholamreza Niknam[1], Edwin Lewis[2], [1]University of Tabriz, Iran, Tabriz, IR; [2]Department of Nematology, University of California, Davis , California, US.

N-13 **The new methods for study entomoparasitic nematodes in Georgia**. Mariam Chubinishvili, Irina Rijamadze, Leven Ninua, Kanchaveli L. Institute of Plant Protection, Tbilisi, GE.

N-14 **The susceptibility entomopatogenic nematode towards the mulberry moth.** Nona Mikaia, Manana Kakhadze, Rusudan Skhirtladze, Tsisia Chkhubianishvili, Kanchaveli L. Institute Plant Protection, Tbilisi, GE.

N-15 **Effect of copper on the flour beetle *Tribolium castaneum* resistance to the entomopathogenic nematode *Steinernema feltiae*.** Paulina Kramarz, Anna Mordarska, Magdalena Mroczka, Jagiellonian University, Krakow, PL.

N-16 **The efficacy of *Steinernema feltiae* and *Steinernema carpocapsae* in controlling Colorado potato beetle on potato under field conditions**. Timea Toth[1], Ziga Laznik[2], Stanislav Trdan[2], Tamas Lakatos[1], [1]Nemaform Ltd., Nagykallo, HU; [2]University of Ljubljana, Ljubljana, SI.

N-17 **STU Interactions between entomopathogenic nematodes (Steinernematidae, Heterorhabditidae) and the citrus nematode *Tylenchulus semipenetrans* (Tylenchulidae) in Arizona.** Patricia Navarro[1], Chan Maketon[2], S. Patricia Stock[1], [1]Department of Entomology, University of Arizona, Tucson, US; [2]Department of Plant Pathology, Washington State University, Pullman, US.

N-18 **New insights in the pathogenic process of *Steinernema carpocapsae*: The role of proteases** Nelson Simões, Duarte Toubarro, Balasubramanian Natesan, Gisela Nascimento, Jing Yingjun, Hao YouJin, Rafael Montiel, University of Azores, Ponta Delgada, PT.

Tuesday July 13, 2010

Osman Turan Congress Center

06:00 Bus pick up at hotels for 5K Runners

06:30 5K Fun Run/ Walk Stadium

07:30 Bus pick up at hotels

Symposium Viruses-1

Tuesday, 8:00-10:00
HasanTuran

Application of Insect Viruses in Medicine

Organizers: Linda King and Monique van Oers

8:00 **45 Opportunities and challenges in medical application of baculovirus**. Monique M. van Oers[1], Linda A. King[2], [1]Laboratory of Virology, Wageningen University, Wageningen, NL; [2]School of Life Sciences, Oxford Brookes University , Oxford, GB.

8:30 **46 A fast track influenza vaccine made in insect cells**. Manon Cox, Protein Sciences Corp, Meriden, US

9:00 **47 Humanised baculoviruses for cancer gene therapy.** Norman Maitland, Guillemo Rivera-Gonzales, Stephanie Swift, Bryn Davies, Lindsay Georgopoulos, YCR Cancer Research Unit, Dept of Biology, University of York, York, GB.

9:30 **48 Novel approaches in producing adeno-associated virus in insect cells**. Cristelle Rivière, Nicolas Laroudie, Martin Marek, Loic Millot, Lionel Galibert, Mehdi Gasmi, Otto-Wilhelm Merten, Généthon, Evry, FR.

Symposium Nematodes

Tuesday, 8:00-10:00
Nihat Turan 1

Biotic and Abiotic Determinants of Entomopathogenic Nematodes: Where Have All the Nematodes Gone?

Organizers: Ed Lewis and Selcuk Hazir

8:00 **49 Persistence on a large scale: Soil type, predators and alternate hosts.** ***Ed Lewis*** University of California Davis, Davis, CA, US.

8:30 **50 Efficacy and persistence of entomopathogenic nematodes for controlling white grubs in peanut fields in Israel**. ***Itamar Glazer[1], David Ben-Yakir[2], Arnon Allouche[3], Oren Buchshtab [4],*** [1]Nematology Div., Agricultural Research Organization (ARO), The Volcani Center, Bet Dagan, IL; [2]Department of Entomology, Agricultural Research Organization (ARO), The Volcani Center, Bet Dagan, IL; [3]BioBee Sde Eliyahu Ltd. Kibutz Sde Eliyahu, Bet She'an Valley, Kibutz Sde Eliyahu, IL; [4]Hevel ma'on Enterprises, Kibutz Magen, IL.

9:00 **51 Potential negative effects of *Sancassania polyphyllae* (Acari: Acaridae) on entomopathogenic nematodes**. ***Zeynep Ipek Ekmen[1], Ibrahim Cakmak[2], Selcuk Hazir[3],***

Mehmet Karagoz[2], Nurdan Ozer[4], Harry K. Kaya[5], [1]Hacettepe Univ. Faculty of Science, Department of Biology, Ankara, TR; [2]Adnan Menderes Univ. Faculty of Agriculture, Department of Plant Protection, Aydin, TR; [3]Adnan Menderes Univ. Faculty of Arts and Science, Department of Biology, Aydin, TR; [4]Hacettepe Univ. Faculty of Science, Department of Biology, Ankara, TR; [5]University of California, Department of Nematology, Davis , CA, US.

9:30 **52 Do arthropod scavengers consume nematode-killed insects? *Baris Gulcu[1], Selcuk Hazir[1], Harry Kaya [2].*** [1]Adnan Menderes University, Aydin, TR; [2]University of California, Dept. of Nematology, Davis, CA, US.

Contributed Papers

Tuesday, 8:00-10:00
Fahri Kuran

FUNGI 1

Chairs: Richard Humber and Ali Sevim

8:00 **53 Study on the characteristics and pathogenicity of the entomopathogenic fungus *Beauveria bassiana* as biological control agent of *Bemisia tabaci*.** Aref Olleka, Ren Shun-xiang, Laboratory of Biological Control, College of Natural Resources and Environment, South China Agricultural University, CN.

8:15 **54 Back to biology: Fungal pathogens in mosquito management**. Nancy Beckage, University of California-Riverside, Riverside, US.

8:30 **55 Research on biological efficiency of entomopathogenic fungi *Fusarium subglutinans* against *Aphis fabae* (Hemiptera: Aphididae)**. Serife Evrim Arici, Ibrahim Golmez, Hasan Demirekin, Hatice Zahmakiran, Ismail Karaca, Suleyman Demirel University, Isparta, TR.

8:45 **56 Comparison of formulations of entomopathogenic fungi for treatment of artificial hideouts for biocontrol of *Cydia pomonella* and *Cydia funebrana*.** Dietrich Stephan, Melanie Herker nn, Darmstadt, DE

9:00 **57 Entomopathogenic fungi as potential biological agents for the control fall webworm – *Hyphantria cunea* Drury (Lepidoptera: Arctiidae)**. Medea Burjanadze, Vasil Gulisashvili Forest Institute, Tbilisi, GE.

9:15 **58 Development of Met52 for the control of sucking insect pests in North America and Europe**. Jarrod Leland, Novozymes Biologicals, Salem, US

9:30 **59 The inhibitory effect of the fungal toxin, destruxin-A, on behavioural fever in the desert locust, *Schistocerca gregaria*.** Vicky Hunt, Gary Lock, Keith Charnley, University of Bath, Bath, GB.

Contributed Papers

Tuesday, 8:00-10:30
Nihat Turan 2

MICROBIAL CONTROL 1

Chairs: O.P. Perera and Ralf-Udo Ehlers

8:00 **60 Toxicity of the *Yersina entomophaga* super toxin complex (YeSTc) on midgut cells of *Costelytra zealandica* (Coleoptera: Scarabaeidae)**. Sean Marshall[1], Heather Gatehouse[2], John Christeller[2], Richard Walls[1], Duane Harland[1], Sandra Jones[1], Trevor Jackson[1], Mark Hurst[1], [1]AgResearch, Lincoln, NZ; [2]Plant and Food Research, Palmerston North, NZ.

8:15 **61 Three new strains of *Bacillus sphaericus* as potential Biocontrol Agent against Mosquitoes**. Muge Yazici[1], Ali Umman Dogan[1] , Gulin Boztas[1], Ertugrul Kilic[1], Huseyin Cetin[2], Sevilhan Mennan[3], Fikrettin Sahin[1], [1]Yeditepe University, Department of Bioengineering, Kayisdagi, Istanbul, TR; [2]Akdeniz University, Antalya, TR; [3]Ondokuz Mayıs University, Samsun, TR.

8:30 **62 Effect of *Bacillus thuringiensis* as vegetative form on hemocytes of *Rhynchophorous ferrugineus* (Coleoptera: Curculionidae) larvae**. Barbara Manachini, Daniela Parrinello, Vincenzo Arizza. Dipartimento di Biologia Animale "G. Reverberi", University of Palermo, Palermo, IT.

8:45 **63 Identification, tissue expression patterns, and genomic structure of alkaline phosphatase genes in the corn earworm, *Helicoverpa zea*.** Omaththage P. Perera[1], Juan Luis Jurat-Fuentes[2], Carlos Blanco[3], [1]SIMRU, USDA-ARS, Stoneville, US; [2]University of Tennessee, Knoxville, US; [3]USDA-APHIS, Washington, DC, US.

9:00 **64 Use of *Bacillus thuringiensis* subsp. *israelensis* for control of the European crane fly *Tipula paludosa* (Diptera: Nematocera)**. Ralf-Udo Ehlers, Institute for Phytopathology, Univ. Kiel, Kiel, DE.

9:15 **65-STU Characterization of insects intracellular response to *Bacillus thuringiensis* Cry toxins**. Angeles Cancino-Rodezno[1], Cynthia Alexander[1], Juan-Luis Jurat[2], Yannik Pauche[3], Ivonne Castro[4], Sabino Pacheco[1], Alejandra Bravo[1], Roberto Villase[1], Humberto Lanz[4], Sarjeet Gill[5], Mario Soberón[1], [1]IBT UNAM, Cuernavaca, MX; IBT UNAM, Cuernavaca, MX; [2]University of Tennessee, Knoxville, US; [3]Universidad of Exter, GB; [4]INSP, Cuernavaca, MX; [5]University of California, US.

9:30 **66 Long-term benefits of GM crops: Potential for *Diabrotica* suppression in Europe using *Bt* maize**. Nicholas Storer[1], Peter Schlotter[2], Kevin Steffey[1], William Hutchison[3], [1]Dow AgroSciences, Indianapolis, US; [2]Dow AgroSciences, Munich, DE; [3]University of Minnesota, St. Paul, US.

9:45 **67 Cross-resistance of *Bt*-resistant *Helicoverpa armigera* (Lepidoptera: Noctuidae) to *Bacillus thuringiensis* Vip3Aa protein**. Qian Zhang, Lizhen Chen, Qiong Lu, Jie Zhang, Gemei Liang, Yuyuan Guo, State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, P.R. China, Beijing, CN.

10:00 **68-STU Analysis of *Manduca sexta* aminopeptidase N1 and alkaline phosphatase as receptors of Cry1Ab toxin by gene silencing**. Biviana Flores-Escobar, Alejandra Bravo, Mario Soberón, Isabel Gómez, Instituto de Biotecnología UNAM, Cuernavaca, Morelos, MX.

10:15 **69 Oenocytoid cell lysis to release prophenoloxidase is induced by eicosanoid via PKC pathway.** Sony Shrestha, Yonggyun Kim. Department of Bioresource Sciences, Andong National University, Andong 760-749, Korea

08:00-10:30 **TAKE POSTERS DOWN**

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

Contributed Papers

Tuesday, 10:30-12:30
Hasan Turan

VIRUSES 3

Chairs: Peter Krell and Primitivo Caballero

10:30 **70 *pif1* expression determines the transmissibility of the *Spodoptera frugiperda* multiple nucleopolyhedrovirus**. Oihane Simón[1], Trevor Williams[2], Martine Cerutti[3], Primitivo Caballero[1], Miguel López-Ferber[4], [1]Instituto de Agrobiotecnología, CSIC-Gobierno de Navarra, 31192, Mutilva Baja, Navarra, ES; [2] Instituto de Ecologia AC, 91070, Xalapa, Veracurz, MX; [3] Laboratoire Baculovirus et Thérapie, CNRS, 30380, Saint Christol-Les-Alés, FR; [4]Laboratoire de Génie de l'Environnement Industriel, Ecole de Mines d'Alès, 30319 , Alés, FR.

10 :45 **71-STU Baculovirus photolyases are DNA repair enzymes with circadian clock regulatory function.** Magdalena Anna Biernat[1], Andre Eker[2], Monique van Oers[1], Just Vlak[1], Gijsbertus van der Horst[2], Ines Chaves[2], [1]Laboratory of Virology, Wageningen Univeristy, Wageningen, NL; [2]Department of Genetics, Erasmus University Medical Center, Rotterdam, NL

11:00 **72-STU A mechanism for AcMNPV proV-CATH retention in the endoplasmic reticulum.** Jeffrey Hodgson[1], Basil Arif[2] , Peter Krell[1]. [1]University of Guelph, Guelph, CA; [2]Great Lakes Forestry Centre, Guelph, CA.

11:15 **73-STU *Mamestra configurata* nucleopolyhedrovirus enhancin substrate specificity and insect intestinal mucin structural types**. Umut Toprak[1], Doug Baldwin[2], Dwayne Hegedus[2], Cedric Gillott[1], Stephanie Harris[2], David Theilmann[3], Martin Erlandson[2], [1]University of Saskatchewan, Saskatoon, CA; [2]Agriculture and Agri-Food Canada, Saskatoon, CA; [3]Agriculture and Agri-Food Canada, Summerland, CA.

11:30 **74 N-terminal of VP3 of *Dendrolimus punctatus* cypovirus plays an essential role in attaching to its host cells.** Liang Jin, Jinjin Liu, Xiulian Sun, State Key Laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, CN.

11:45 **75 Genome sequence of a granulovirus occlusion body shape/size mutant.** Shohei Hikihara , Rie Ukuda, Yasuhisa Kunimi, Ayako Hirao, Madoka Nakai, Tokyo University of Agriculture and Technology, Tokyo, JP.

12:00 **76 Formation of few polyhedra (FP) by AcMNPV 25K FP mutants is dependent on cell lines**. Xin-Huan Cheng, Senthil Kumar, Jianli Xue, Xiao-Wen Cheng. Miami University, Oxford, US.

12:15 **77 A silencing suppressor protein (NSs) of a tospovirus enhances baculovirus replication in permissive, semipermissive and nonpermissive insect cell lines.** Virgínia Carla Oliveira[1], Lorrainy Bartasson[1], Maria Elita Batista Castro[2], Jose Raimundo Correa1, Bergmann Morais Ribeiro[1] and Renato de Oliveira Resende[1], [1]University of Braslia, Brasilia, BR; [2]Embrapa Recursos Geneticos e Biotecnologia, Brasilia, BR.

Contributed Papers

Tuesday, 10:30-12:30
Fahri Kuran

FUNGI 2

Chair: Jarrod Leland and Ismail Karaca

10:30 **78 Mechanism of long-term suppression of a forest pest by application of *Beauveria bassiana*.** Zengzhi Li, Bin Wang, Anhui Agricultural University, Hefei, Anhui, 230036, CN.

10:45 **79 Transmission of *Metarhizium anisopliae* between male and female Asian longhorned beetles, *Anoplophora glabripennis***. Fan Peng[1], Sana Gardescu[2], Ann Hajek[2], [1]Work conducted while visiting Cornell University Ithaca, New York, US; (presently a student at Anhui Agricultural University), [2]Cornell University, Ithaca, New York, US.

11:00 **80 Occurrence of entomopathogenic fungi in soils from different habitats in Poland**. Cezary Tkaczuk, Department of Plant Protection, University of Podlasie, Siedlce, PL.

11:15 **81 Identification, isolation and virulence of entomopathogenic fungi collected from cereal aphids in Argentina and South Africa**. Lopez Lastra, Claudia C.[1], Hatting Justin[2], [1] CEPAVE, La Plata, AR; [2]ARC-Small Grain Institute, P/Bag X29, Bethlehem 9700, South Africa, Bethlehem, ZA.

11:30 **82 Culture of *Metarhizium robertsii* on salicylic-acid supplemented media induces increased conidial thermotolerance, but not UV-B tolerance**. Drauzio E. N. Rangel[1], Éverton K. cK. Fernandes[2], Donald W. Roberts[2], [1]Universidade do Vale do Paraiba, Sao Jose dos Campos, BR; [2]Utah State University, Logan, US.

11:45 **83 Effect of temperature and time of exposure on the viability and virulence of *Beauveria bassiana* and *Metarhizium anisopliae* in dried unformulated conidia and formulated conidia**. Oliveira Daian G. P, Alves Sérgio B. Delalibera Jr. Italo, ESALQ-USP, Piracicaba-SP, BR.

12:00 **84 Effect of light intensity and time of exposure on sporulation and germination of *Neozygites floridana***. Vitalis Wafula Wekesa[1], Thiago Rodrigues Castro[2], Ingeborg Klingen[1], Italo Delalibera Jr[2], [1]Nowegian Institute for Agricultural and Environmental Research (Bioforsk), Plant Health and Plant Protection Division, Department of Entomology and Nematology, Høgskoleveien 7, N-1432 Ås, Norway., Ås, NO; [2]Department of Entomology and Acarology, Laboratory of Pathology and Microbial Control of Arthropods; ESALQ-USP; 63418-900 Piracicaba-SP; Brazil., Piracicaba, BR.

12:15 **85-STU Cold-seeking behaviour in *Drosophila* during mycosis: consequences for host and pathogen**. Vicky Hunt, Keith Charnley, Nick Priest, University of Bath, Bath, GB.

Contributed Papers

Tuesday, 10:30-12:30
Nihat Turan 1

MICROBIAL CONTROL 2

Chairs: Jean Maniania and Reza Talaei

10:30 **86-STU Effects of the presence of susceptible and non-susceptible insects on *Beauveria bassiana* F418 gfp tr3 persistence in soil**. Céline Blond[1], Travis Glare[1], Hayley Ridgway[2], Bruce Chapman[3],

Leo Condron[2], Michael Brownbridge[4], [1]Bio-Protection Research Centre, Lincoln University, Lincoln, NZ; [2]Agriculture and Life Sciences, Lincoln University, Lincoln, NZ; [3]Insect Science Ltd, Christchurch, NZ; [4]Vineland Research and Innovation Centre, Ontario, CA.

10:45 **87-STU Establishment of the fungal entomopathogen *Beauveria bassiana* (Ascomycota: Hypocreales) as an endophyte in broad bean and oilseed rape and its potential for insect biocontrol**. Lara R. Jaber[1], Helge Stahlmann[1], Tadele Tefera[2], Stefan Vidal[1], [1]Georg-August-University Goettingen, Göttingen, DE; [2]International Maize and Wheat Improvement Center (CIMMYT), Nairobi, KE.

11:00 **88 Effect of soil moisture and texture on virulence of entomopathogenic fungi**. Ali Derakhshan, Shahrood university of technology, Shahrood, IR.

11:15 **89 Field efficacy of the *Metarhizium anisopliae* isolate ICIPE78 in controlling the red spider mite *Tetranychus evansi* in tomato field crop in Central Kenya**. David Mogisho Bugeme[1], Nguya Kalemba Maniania[1], Adenirin Chabi-Olaye[1], Hamadi Iddi Boga[2], Markus Knapp[1], [1]International Centre of Insect Physiology and Ecology (icipe), Nairobi, KE; [2]Faculty of Sciences, Jomo Kenyatta University of Agriculture and Technology , Nairobi, KE.

11:30 **90 Can *Metarhizium anisopliae* treated semiochemical-baited traps reduce *Amblyomma variegatum* populations in the field?** Felix Nchu[1,2], Nguya Kalemba Maniania[1], Ahmed Hassanali[1], Kobus Eloff[2], [1]International Centre of Insect Physiology and Ecology (icipe), Nairobi, KE; [2]Programme for Phytomedicine, Department of Paraclinical Sciences, University of Pretoria, Pretoria, ZA.

11:45 **91 Synergistic effect of dual imidacloprid *Metarhizium anisopliae* applications against Asian longhorned beetles (*Anoplophora glabripennis*)**. Todd Ugine, Calum Russell, Ann Hajek, Cornell University, Ithaca, US.

12:00 **92 Origin and spreading track of white muscardine of silkworms.** Zengzhi Li, Jiali Li, Fenggang Luan, Anhui Agricultural University, Hefei, Anhui 230036, CN.

12 :15 **93 Nuclear polyhedrosis virus (NPV) infection counters insecticide resistance in *Helicoverpa armigera***. Anwaar Alvi[1], Ali Sayyed[2], [1]PMAS Arid Agriculture University, Rawalpindi, PK; [2]Bahaudin Zakariya University, Multan, PK.

12 :30 **94 Baculovirus - how much is a lethal concentration?** Sean Moore[1], Lyndall Pereira da Conceicoa[2], Wayne Kirkman[1], Martin Hill[3], Stephan Verreyne[4], Paul Fourie[4], [1]Citrus Research International, Port Elizabeth, ZA; [2]Du Roi IPM, Letsitele, ZA; [3]Rhodes University, Grahamstown, ZA; [4]Citrus Research Internatinal, Stellenbosch, ZA.

Contributed Papers

Tuesday, 10:30-12:30
Nihat Turan 2

MICROSPORIDIA 1

Chair: David Oi and Kubilay Er

10:30 **95 Rapid build up of *Nosema fumiferanae* in outbreak populations of the jack pine budworm, *Choristoneura pinus pinus*.** Kees van Frankenhuyzen, Yuehong Liu, Canadian Forest Service, Sault Ste. Marie, CA.

10:45 **96 Occurrence of the microsporidium *Canningia tomici* and artificial infection in the pine shoot beetles *Tomicus piniperda* and *Tomicus minor* (Coleoptera: Scolytidae)**. Rudolf Wegensteiner, ***Dorte Gortz,*** Milan Pernek, Uwe Handel, Jaroslaw Weiser, Boku University Vienna, Vienna, AT.

11:00 **97 Molecular phylogenetics of *Thelohania muelleri* like parasites infecting gammarid amphipods.** Toby Wilkinson[1], Martin Kamler[2], Joseph Ironside[1], [1]Aberystwyth University, Aberystwyth, GB; [2]University of Veterinary and Pharmaceutical Sciences Brno, Brno, CZ.

11:15 **98 Molecular phylogeny of five microsporidian species infecting *Chironomus plumosus* (Diptera: Chironomidae) in North-Western Russia**. Yuri Tokarev[1], Vladimir Voronin[2], Irma Issi[1], [1]All-Russian Institute of Plant Protection, St. Petersburg-Pushkin, RU; [2]State Research Institute of Lake and River Fisheries, St. Petersburg, RU.

11:30 **99-STU Discovery of a novel microsporidian infecting commercial cultures of the Mediterranean cricket *Gryllus bimaculatus*.** Katy Peat**,** Joseph Ironside, Institute of Biological, Environmental and Rural Sciences, Aberystwyth, GB .

11:45 **100 Caught in the crossfire: Mismatch between morphological and molecular taxonomic data in classification of a novel microsporidian parasite of lobsters.** Grant Stentiford[1], Kelly Bateman[1], Hamish Small[2], Jessica Moss[2], Jeffrey Shields[2], Kim Reece[2], Ian Tuck[3], [1]Cefas, Weymouth, GB; [2]VIMS, Virginia, US; [3]NIWA, Auckland, NZ.

12:00 **101 PCR identification, phylogeny analysis of *hsp*70 gene of insect microsporidia**. Ji-Ping Liu, Jing_Xia Li, South China Agriculture University, Guangzhou, CN.

12:15 **102 Intragenomic diversity of ribosomal DNA in *Nosema***. Joseph Ironside, Aberysytwyth University, Aberystwyth, GB.

12:30-13:45 LUNCH at KTU SAHIL

14:00-21:30 EXCURSION AND BBQ

18:00-21:30 BBQ. Buses returns to hotels

Wednesday July 14, 2010

Osman Turan Congress Center

07:30 Bus pick up at hotels

08:00-09:30 Posters Up
Bacteria, COST 862, Viruses and Fungi

Symposium Bacteria

Wednesday, 8:00-09:30
Hasan Turan

Insecticidal Products from Bacterial Genome Sequencing.

Organizer: Neil Crickmore

8:00 **103 The diversity of insecticidal toxins from bacteria.** Daniel R. Zeigler[1], Vidisha Krishnan[2], Neil Crickmore[2] [1]Bacillus Genetic Stock Center,

Department of Biochemistry, The Ohio State University, Columbus, OH, US; [2]School of Life Sciences, University of Sussex, Brighton, UK.

8:30 **104 Pesticidal gene discovery using *de novo* sequencing of bacterial genomes**. Kimberley Sampson, Athenix - a Bayer CropScience business, Research Triangle Park, NC, US.

9:00 **105 The *Photorhabdus* genome as a source of novel insecticidal products**. Maria Sanchez-Contreras[1], Andrea Dowling[2], Paul Wilkinson[2], Federico Dorati[3], Robert Jackson[3], Richard H. ffrench-Constant[2], Nick R. Waterfield[1], [1]Department of Biology and Biochemistry, University of Bath, UK; [2]Department of Biological Sciences, University of Exeter, UK; [3]Department of Microbiology, University of Reading, UK.

Symposium Microsporidia

Wednesday, 8:00-10:30
Nihat Turan 1

Microsporidia and Other Pathogens in Arthropods from the Eastern Mediterranean Region.

Organizer: Andreas Linde

8:00 **106 Release** of ***Nosema lymantriae, Vairimorpha disparis* and *Entomophaga maimaiga* for classical and augmentative biological control of gypsy moth in Bulgaria and the United States.** Daniela Pilarska[1], Andreas Linde[2], Plamen Pilarski[3], Danail Takov[1], Georgi Georgiev[4], Leellen Solter[5], [1]Institute of Zoology, BAS, Sofia, BG; [2]University of Applied Sciences, Eberswalde, DE; [3]Institute of Plant Physiology, BAS, Sofia, BG; [4]Forest Research Institute, BAS, Sofia, BG; [5]Illinois Natural History Survey, University of Illinois, Champaign, US.

8:25 **107 Pathogens of forest pest insects in Georgia**. Manana Kereselidz[1], Daniela Pilarska[2], Nana Goginashvili[1], [1]Vasil Gulisashvili Forest Institute, Tbilisi, GE; [2]Institute of Zoology, Sofia, BG.

8:50 **108 An overview on entomopathogenic protists from Turkey**. Andreas Linde, University of Applied Sciences, Eberswalde, DE.

9:15 **109 Pathogen infections of the bark beetle *Dendroctonus micans* and its predator *Rhizophagus grandis* from Turkey**. Renate Radek, Free University of Berlin, Berlin, DE.

9:40 **110 Entomopathogenic fungi as potential microbial control agents against hazelnut and forest pests in the Black Sea region of Turkey.** Ali Sevim, Ismail Demir, Elif Tanyeli, Zihni Demirbag, Karadeniz Technical University, Faculty of Arts and Sciences, Department of Biology, Trabzon, TR.

10:05 **111 Release of *Nosema lymantriae, Vairimorpha disparis* and *Entomophaga maimaiga* for classical and augmentative biological control of gypsy moth in Bulgaria and the United States**. Daniela Pilarska[1], Andreas Linde[2], Plamen Pilarski[3], Danail Takov[1], Georgi Georgiev[4], Leellen Solter[5], [1]Institute of Zoology, BAS, Sofia, Bulgaria; [2]University of Applied Sciences, Eberswalde, Germany, [3]Institute of Plant Physiology, BAS, Sofia, Bulgaria, [4]Forest Research Institute, BAS, Sofia, Bulgaria, [5]Illinois Natural History Survey, University of Illinois, Champaign, IL,USA.

Contributed Papers

Wednesday, 8:00-10:00
Fahri Kuran

VIRUSES 4

Chair: Bob Harrison and Nor Chejanovsky

8:00 **112 RNase III expressed by ascoviruses and its potential roles in host-virus interactions.** Mazhar Hussain, Sassan Asgari, The University of Queensland, Brisbane, AU.

8:15 **113-STU AcMNPV IE-1 plays an important role in the development of virogenic stroma in the baculovirus life cycle**. Yang Liu, Meijin Yuan, Kai Yang, Yi Pang, State Key Lab of Biocontrol, Sun Yat-sen University, Guangzhou, CN.

8:30 **114 Strong activation of viral genes in mammalian cells by baculovirus IE2 depends on a novel viral nuclear structure.** Catherine Liu,Yu-Chen Chao Institute of Molecular Biology, Academia Sinica, Taipei, TW.

8:45 **115 Identification and mutagenesis of cysteine residues which play critical roles in the formation of intersubunit disulfide bridge of mature d F protein of *Helicoverpa armigera* single nucleocapsid nucleopolyhedrovirus.** Feifei Yin[1], Manli Wang[1], Ying Tan[1], Fei Deng[1], Just M. Vlak[2], Zhihong Hu[1], Hualin Wang[1], [1]State Key Laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, CN; [2]Laboratory of Virology, Wageningen University, Wageningen, NL.

9:00 **116 AcMNPV ME53 co-localizes with GP64 at viral budding sites.** Jondavid de Jong[1], David Theilmann[2], Basil Arif[3], Peter Krell[1], [1]University of Guelph, Guelph, CA; [2]Pacific Agri-Food Research Centre, Summerland, CA; [3]Great Lakes Forestry Centre, Sault Ste Marie, CA.

9:15 **117 *Autographa californica* multiple nucleopoly hedrovirus ac76 is involved in intranuclear microvesicle formation**. Zhaoyang Hu, Meijin Yuan, Wenbi Wu, Chao Liu, Kai Yang , Yi Pang, State Key Lab of Biocontrol, Sun Yat-sen University, Guangzhou, CN.

9:30 **118 A non-coding RNA of HzNV-1 virus establishes latent viral infection through micro RNA**. Yueh-Lung Wu, Yu-Chan Chao, Institute of Molecular Biology, Academia Sinica, ROC, Taipei, TW.

Contributed Papers

Wednesday 8:00-10:00
Nihat Turan 2

NEMATODES 1

Chairs: Ed Lewis and Selcuk Hazir

8:00 **119 Genes that are involved in the recovery process in the entomopathogenic nematode *Heterorhabditis bacteriophora***. Anat Moshayov[1], Hinanit Koltai[2], Itamar Glazer[1], [1]Department of Entomology, Nematology Div Bet Dagan 50250, IL; [2]Depart. of Ornamental Horticulture, ARO, Agricultural Research Organization, The Volcani Center, P.O. Box 6, Bet Dagan 50250, IL

8:15 **120 Exploring the molecular basis of 'Recovery' in infective juveniles of the entomopathogenic nematode *Heterorhabditis bacteriophora* TTO1**. Glazer Itamar[1], Moshayov Anat[1], Koltai Hinanit[2], [1]Nematology Div., Agricultural Research Organization (ARO), The Volcani Center, Bet

Dagan, IL; [2]Depart. of Ornamental Horticulture, Agricultural Research Organization (ARO), The Volcani Center, Bet Dagan, IL.

8:30 **121 *Steinernema* nematodes and their bacterial endosymbionts: A multigene approach to inferring their evolutionary histories**. Ming-Min Lee[1], S. Patricia Stock[2], [1]Department of Entomology, University of Arizona, Tucson, US; [2]Depart of Plant Pathology, Washington State University, Tucson, US.

8:45 **122 Diversity, distribution, and phylogenetic analysis as inferred from ribosomal DNA sequences of the ITS1-5.8s-ITS2 region of entomopathogenic nematodes in Pakistan.** Mehreen Gulsher Gulsher, Shahina Fayyaz Fayyaz, National Nematological Research Centre, Karachi, PK.

9:00 **123 An outlook on Italian EPN biodiversity**. Eustachio Tarasco[1], Mirella Clausi[2], Tiziana Panzavolta[3], Giancarlo Rappazzo[2], Pasqua Vernile[1], Agata Longo[2], Diego Leone[2], Riziero Tiberi[3], Marisa Vinciguerra[2], Oreste Triggiani[1], [1]Dipartimento di Biologia e Chimica Agro-Forestale e Ambientale (DiBCA), Università degli Studi di Bari "Aldo Moro". Bari, IT; [2]Dipartimento di Biologia Animale, Università degli Studi di Catania, Catania, IT; [3]Dipartimento di Biotecnologie Agrarie, Università degli Studi di Firenze, Firenze, IT; Giovanna Curto - Servizio Fitosanitario dell'Emilia Romagna, Bologna, IT.

9:15 **124 Novelty in distribution of entomopathogenic nematodes**. Zdenek Mracek, Vladimir Puza, Biology Centre, Institute of Entomology Czech Academy of Sciences, Ceske Budejovice, Czech Republic, Ceske Budejovice, CZ.

9:30 **125-STU Development of a cultivated system with insect cells for an entomopathogenic nematode *Steinernema carpocapsae*, and analysis of the recovery**. Shingo Kikuta, Takashi Kiuchi, Masao Nagata, Graduated School of Frontier Sciences, The University of Tokyo; National Institute of Agrobiological Sciences. Chiba, JP.

9:45 **126 Diversity and biogeographic distribution of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in Lebanon.** Noujeim E; Khater C; Pages S; Ogier JC; Taillez P; Hamze M; Thaler O.

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

Symposium Viruses

Wednesday 10:30-12:30
Hasan Turan

Arthropod Transmitted Viral Diseases

Organizers: Hu Zhihong (Rose) and Gorben Pijlman

10:30 **127 CCHFV-host interaction and the current situation in Turkey**. Zati Vatansever, Kafkas University, Kars, TR.

11:00 **128 Chikungunya, a threat for Europe? Opinion from an entomologist**. Anna-Bella Failloux, Institut Pasteur, Paris, FX.

11:30 **129 Detection of Semliki forest virus infection and Induction of antiviral responses by the mosquito innate immune system.** Alain Kohl, The Roslin Institute, University of Edinburgh, Edinburgh, GB.

12:00 **130 West Nile virus-host interactions: The poison is in the tail.** Gorben Pijlman, Laboratory of Virology, Wageningen University, Wageningen, NL.

Symposium Diseases of Beneficial Invertebrates

Wednesday 10:30-12:30
Nihat Turan 2

Pathogens of Pollinators: A Molecular Perspective

Organizer: Elke Genersch

10:30 **131 Transcriptome analysis of honey bee, *Apis mellifera* larvae infected with chalkbrood fungus**. Katherine Aronstein, Daniel Murray, Eduardo Saldivar, USDA/ARS, Weslaco, US.

11:00 **132 *Nosema ceranae* and *Nosema apis* – Comparative virulence.** Ingemar Fries, Swedish University of Agricultural Sciences, Uppsala, SE.

11:30 **133 European foulbrood in the molecular era**. Eva Forsgren, Institutionen för ekologi, Department of Ecology SLU, Swedish University of Agricultural Sciences, P.O.Box 7044, SE-75007, Uppsala, SE.

12:00 **134 Molecular analysis of the honey bee pathogen *Paenibacillus larvae*.** Elzbieta Brzuszkiewicz, Institute of Microbiology and Genetics, University of Goettingen, Goettingen, DE.

Contributed Papers

Wednesday, 10:30-12:30
Fahri Kuran

FUNGI 3

Chairs: Drauzio Rangel and Ozlem Kalkar

10:30 **135 Changing perspectives on the Entomophthorales: A new look at some of the oldest fungi**. Richard A. Humber[1], Bo Huang [2], Andrii J. Gryganskyi[3], Rytas Vilgalys[3], Kathie Hodge[4], [1]USDA-ARS BioIPM Research, RW Holley Center for Agriculture and Health, Tower Road, Ithaca, New York, US; [2]Anhui Agricultural University, Hefei, CN; [3]Duke University, Durham, North Carolina, US; [4]Cornell University, Ithaca, New York, US

10:45 **136 Experiences in development of *Beauveria bassiana* for use in the IPM pest scarabs in Mexico.** Miguel B. Nájera Rincón[1], Trevor A. Jackson[2], [1] Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), MX, [2]AgResearch Ltd. Lincoln Research Centre, NZ.

11:00 **137 The ATP-driven efflux pump is involved in *Isaria fumosorosea* resistance to carbendazim**. Ting-tingSong, Sheng-hua Ying, Ming-guang Feng, Zhejiang University, Hangzhou, CN.

11:15 **138 Characterization of a new mitochondrial Mn-SOD (BbSod3) from *Beauveria bassiana***. Xue-qin Xie, Sheng-hua Ying, Ming-guang Feng, Zhejiang University, Hangzhou, CN.

11:30 **139Analysis of a trehalose-6-phosphate synthase gene cloned from *Beauveria bassiana***. Qian Liu, Sheng-hua Ying, Ming-guang Feng, Zhejiang University, Hangzhou, CN.

11:45 **140-STU The effect of the volume of medium on the growth and conidiation of *Pandora heteropterae* (Entomophthoraceae: Entomophthorales)**. Joshua Hannam, Donald Steinkraus, University of Arkansas, Fayetteville, US.

Wednesday, 10:30-12:30

VIEW POSTERS
Bacteria and COST 862
(Authors stand by posters when not in session).

12:30-14:00 **LUNCH at KTU SAHIL**

13:00-14:00 **JIP Board Meeting** Senate Hall

Cross Divisional Symposium

Wednesday, 14:00-16:00
Hasan Turan

Viruses and Diseases of Beneficial Invertebrates

Viruses of Pollinators

Organizers: Nancy Ostiguy and Ivan Meeus

14:00 **141 Multiplex detection of viruses in bumblebees**. Meeus Ivan[1], Smagghe Guy[1], Jans Kris[2] and de Graaf Dirk[1], [1]Ghent University, Ghent, BE; [2]Biobest, Westerlo, BE.

14:30 **142 Dicistroviruses in Honey Bees: An overview and future research direction**. Joachim de Miranda, Swedish University of Agricultural Sciences, Uppsala, SE.

15:00 **143 DWV – An interesting bee virus**. Sebastian Gisder, Nadine Möckel and Elke Genersch, Institute for Bee Research, Hohen Neuendorf, DE.

15:30 **144 Recent advances in CBPV (Chronic bee paralysis virus) study**. Magali Ribière, Philippe Blanchard, Frank Schurr, V. Olivier, O. Celle, A. Chevin, J. Carletto, Jean-Paul Faucon, Unité pathologie de l'abeille, AFSSA-LERPRA B.P. 111, 06902 , Sophia Antipolis, FR.

Contributed Papers

Wednesday, 14:00-16:00
Fahri Kuran

BACTERIA 3

Chair: Christine Nielsen-Leroux and Ralf-Udo Ehlers

14:00 **145-STU *Bti* is not as safe! Tools for detecting *Bti* persistence and mosquito resistance in the field.** Guillaume Tetreau, Margot Paris, Stéphane Reynaud, Jean-Philippe David, Laboratory of Alpine Ecology (LECA), Grenoble, FX.

14:15 **146-STU *Bti* proliferation in the environment: Impact on the evolution of insecticide resistance**. Margot Paris[1], Aurélie Bonin[1], Guillaume Tetrau[2], Jean-Philippe David[2], Laurence Despres[2], [1]CNRS, Grenoble, FR; [2]Université Joseph Fourier, Grenoble, FR.

14:30 **147 Increase in midgut microbiota load increases tolerance to *Bacillus thuringiensis***. Patricia Hernández-Martínez, Bahram Naseri, Gloria Navarro-Cerrillo, Baltasar Escriche, Juan Ferre, Salvador Herrero, Department of Genetics, Universitat de València , Burjassot, ES.

14:45 **148 Influence of population density of *Xenorhabdus bovienii* and *X. nematophila* on the development of their symbiotic nematodes *Steinernema feltiae* and *S. carpocapsae* in monoxenic liquid cultures.** Ralf-Udo Ehlers[1], Ayako Hirao[2], [1]Inst. Phytopathology, University Kiel, Kiel, DE; [2]University Tokio, Tokio, JP.

15:00 **149 Ultrastructural changes in the gut of adult flies after *Brevibacillus laterosporus* ingestion.** Luca Ruiu[1,2], Alberto Satta[1], Ignazio Floris[1], [1] Department of Plant Protection, University of Sassari, Sassari, IT; [2]Bioecopest srl (Technology Park of Sardinia), Sassari, IT.

15:15 **150 Oral insecticidal activity in biocontrol pseudomonads**. Beat Ruffner[1,] Maria Péchy-Tarr [2], Patrik Hoegger[3], Christoph Keel[2], Maurhofer Monika[1], [1]Plant Pathology, Institute of Integrative Biology, ETH Zurich, Zurich, CH; [2]Department of Fundamental Microbiology, University of Lausanne, Lausanne, CH; [3]Syngenta Crop Protection AG, Stein, CH.

15:30 **151 Insecticidal effect of *Bacillus thuringiensis* Berliner ssp. *tenebrionis* (Bacteria: Bacillaceae), on partially treated wheat and maize surfaces, against larvae of *Tribolium confusum Jacquelin* du Val (Coleoptera: Tenebrionidae), *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) and *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae)**. Christos Athanassiou[1], Nickolas Kavallieratos[2], Basileios Vayias[3], [1]University of Thessaly, Nea Ionia, Magnissia, Greece; [2]Benaki Phytopathological Institute, Kifissia, Attica, Greece; [3]Hellenic Ministry of Rural Development and Food, Athens, Greece .

15 :45 **152 Human exposure to airborne *Bacillus thuringiensis kurstaki* HD1 and other bacteria in greenhouses and vegetable fields**. Vinni Mona Hansen[1], Jørgen Eilenberg[2], Anne Mette Madsen[1], [1]The National Research Centre for the Working Environment, Copenhagen, DK; [2]Copenhagen University, Copenhagen, DK.

Contributed Papers

Wednesday 14:00-16:00
Nihat Turan 2

NEMATODES 2

Chair: Jeanne de Waal and I. Alper Susurluk

14:00 **153 Analysis of community composition, diversity and function of nematodes in the rhizosphere soil of replanted and non-replanted peach orchards**. Qi-zhi Liu, Xiao-yin Du, Na Xie, Hai-ying Zhou, College of Agriculture and biotechnology, China Agricultural University, Beijing, CN.

14:15 **154 Scavenging extends the host range of entomopathogenic nematodes (Nematoda: *Steinernematidae*)**. Vladimir Puza, Zdenek Mracek, Laboratory of Insect Pathology, Institute of Entomology, Czech Academy of Sciences, Branišovská 31, Ceske Budejovice, CZ.

14:30 **155-STU Potential of entomopathogenic nematodes for the control of *Phlyctinus callosus* (Schönherr) (Coleoptera: Curculionidae)** T. Ferreira, A.P. Malan, Stellenbosch University, Department Conservation Ecology and Entomology, P/Bag X1, Matieland, South Africa.

14:45 **156-STU European Earwig (*Forficula auricularia*) as a novel host for the entomopathogenic nematode *Steinernema carpocapsae***. Amanda Hodson, Melissa Moore, Lily Wu, Edwin Lewis, University of California Davis, Davis, US.

15:00 **157 Modifying citrus planting sites promotes conservation biological control of the root weevil *Diaprepes abbreviatus* by entomopathogenic nematodes.** Larry Duncan, Robin J. Stuart, Fahiem El-Borai, Raquel Campos-Herrera, Ekta Pathak, University of Florida, Lake Alfred, FL, US.

15:15 **158-STU The Response of *Phasmarhabditis hermaphrodita* (Nematoda: Rhabditidae) and *Steinernema feltiae* (Nematoda: Steinernematidae) to volatile and water soluble cues**. Jiri Nermut, Vladimir Puza, Zdenek Mracek, Institute of Entomology - Biology Centre AS CR v.v.i., Branisovska 31, 37005 Ceske Budejovice, CZ.

15:30 **159-STU Development time and survivorship of *Deladenus siricidicola* (Tylenchida: Neotylenchidae) on different strains of *Amylostereum areolatum* (Russulales: Stereaceae)**. Erin Morris, Ann Hajek, Alex Navarro, Cornell University, Ithaca, US.

Wednesday, 14:00-16:00

VIEW POSTERS

Fungi (Authors stand by posters when not in session).

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

Contributed Papers

Wednesday, 16:30-18:30
Nihat Turan 1

DISEASES OF BENEFICIAL INVERTEBRATES 1

Chairs: Grant Stentiford and Ibrahim Cakmak

16:30 **160 Specialized parasite (*Varroa destructor*) and hygienic behavior of honey bees (*Apis mellifera*)**. Ibrahim Cakmak, Uludag University, Bursa, TR.

16:45 **161 Assessment of the environmental impact of a "stacked" Bt-maize line with multiple resistances on non-target arthropods.** Eva Schultheis, Alan J.Slusarenko, Stefan Rauschen, RWTH Aachen University, Department of Plant Physiology (Biology III), Aachen, DE.

17:00 **162-STU Potential effects of the *Diabrotica virgifera vir.* specific Cry3Bb1 on the ground beetle *Poecilus cupreus* (L.) evaluated in a full life cycle test.** Kai U. Priesnitz[1], Thomas Thieme[2], Ullrich Benker[1], [1]1Bavarian State Research Center for Agriculture, Freising, DE; [2]Bio-Test Labor Sagerheide, DE.

17:15 **163 RNAi at work: Targeting invertebrate pests and demonstrating effective RNAi protection from pathogenic diseases in honey bees.** Gal Yarden[1], Eitan Glick[1], Ilan Sela[2], Eyal Maori[2], Wayne Hunter[3], Jay Evans[4], Nitzan Paldi[1], Eyal Ben-Chanoch[5], [1]Beeologics, Inc, Rehovot, IL; [2]Hebrew University of Jerusalem Israel, Rehovot, IL; [3]USDA-ARS, Fort Pierce, US; [4]USDA-ARS, Beltsville, US; [5]President and CEO, Beeologics, Miami, Florida US.

17:30 **164 Distribution and variation of *Nosema bombi* in North American bumble bees**. Nils Cordes[1], Leellen Solter[2], [1]University of Illinois, Urbana, US; [2]Illinois Natural History Survey, Champaign, US.

17:45 **165 Involvement of deformed wing virus (DWV) and *Varroa destructor* virus in the deformed wing syndrome of the honey bee.** Nor Chejanovsky, Tzioni Naama, Victoria Soroker, The Volcani Center, Bet Dagan, IL.

Contributed Papers

Wednesday, 16:30-18:30
Nihat Turan 2

NEMATODES 3

Chairs: S. Patricia Stock and Ramazan Canhilal

16:30 **166 Influence of humidity, water application volume and a formulation on the control potential of the entomopathogenic nematode *Steinernema feltiae* on overwintering larvae of the codling moth *Cydia pomonella***. Ralf-Udo Ehlers, Institute for Phytopathology, Univ. Kiel, Kiel, DE.

16:45 **167 Virulence of South Carolinian heterorhabditid isolates to *Spodoptera exigua* (Lepidoptera: Noctuidae)**. Ramazan Canhilal, Erciyes University, Agricultural Faculty, Plant Protection Depart, Kayseri, TR.

17:00 **168 Field trials with entomopathogenic nematodes for the control of false codling moth (*Thaumatotibia leucotreta*)**. Antoinette Malan[1], Sean Moore[2], [1]Depart of Conservation Ecology and Entomology, Faculty of AgriSciences, University of Stellenbosch, South Africa, Stellenbosch, ZA; [2]Citrus Research International, South Africa, Humewood, ZA.

17:15 **169-STU Mulching madness - evaluating the use of mulches in conjunction with entomopathogenic nematodes for the microbial control of codling moth.** De Waal J. Y., A. P. Malan , M. F. Addison, Depart of Conservation Ecology and Entomology, Faculty of AgriSciences, Stellenbosch University, South Africa.

17:30 **170 Evaluation of Pakistani strains of entomopathogenic nematode to some major stored product insect pests**. Salma Javed Javed, Shahina Fayyaz Fayyaz, National Nematological Research Centre, Karachi, PK.

17:45 **171 Current and future prospective of entomopathogenic nematode in Pakistan**. Shahina Fayyaz Fayyaz, National Nematological Research Centre,University of Karachi, Karachi, PK.

Wednesday, 16:30-18:30

VIEW POSTERS

Bacteria, COST 862, Virus and Fungi
(Authors stand by posters).

Wednesday,18:30-20:30
Nihat Usta

DINNER AND AUCTION

Wednesday, 20:30-22:30

SIP DIVISION BUSINESS MEETING

COST 862, MC Meeting

Fahri Kuran

Microbial Control

Business Meeting

Workshop: Pathogens of arthropods other than insects
Chair: Surendra Dara

21:00 **172 Host pathogen interaction between ticks and entomopathogenic fungi.** Glazer Itamar[1], Gindin Galina[1], Ment Dana1, Samish Michael[2], [1]Nematology Div. Agricultural Research Organization (ARO), The Volcani Center , Bet

Dagan, IL; [2]Kimron Veterinary Institute, , Bet Dagan, IL.

21:20 **173 Iridovirus and Rickettsiella infections of isopods**. Brian Federici, University of California, Riverside, Riverside, US.

21:40 **174 Pathogens of predatory mites used for biological pest control**. Susan Bjornson, Saint Mary's University, Halifax, CA.

22:00 **175 Interactions between a large DNA virus (WSSV) and an invertebrate host (shrimp)**. Chu-Fang Lo. National Taiwan University , Taipei, TW.

Nihat Kuran 2

Diseases of Benefical Invertebrates

Business Meeting

Workshop: Histopathology of beneficial invertebrates
Chair: Grant Stentiford

21:00 **176 Histopathology of crustaceans: a frontline tool in pathogen discovery and diagnosis.** Grant Stentiford, Kelly Bateman, European Community Reference Laboratory for Crustacean Diseases, Weymouth, GB.

21:20 **177 Histopathology of bivalve molluscs**. Matt Longshaw, Cefas Weymouth Laboratory, Weymouth, GB.

21:40 **178 Histopathology of bees: A neglected discipline**. Sebastian Gisder, Nadine Möckel, Elke Genersch, Institute for Bee Research, Hohen Neuendorf, DE.

22:00 **179 A dyeing art: Histopathology for the assessment of health and disease in terrestrial insect populations**. Gemma Baron[1], Grant Stentiford[2], Matthew Green[2], Ruth Hicks[2], Helen Roy[3], Helen Hesketh[3], Gabriele Rondoni[4], [1]University of Reading, Reading, GB; [2]Centre for Environment, Fisheries and Aquaculture Science, Weymouth, GB; [3]Centre for Ecology and Hydrology, Wallingford, GB; [4]Perugia University, Perugia, IT.

Demonstration Room

Student and Post Docs Committee Meeting

20:45 **180 How to prepare for an interview**. S. Patricia Stock University of Arizona Tucson , AZ, US.

21:15 **181 Co-operation and collaboration (Liaisons) in thematic research.** Jorgen Eilenberg, Copenhagen University, Copenhagen, DK.

22:45 Buses depart for hotels

POSTERS
Bacteria, COST 862, Virus and Fungi

BACTERIA

B-01 **Study of the bacterial flora as a biological control agent of *Lymantor coryli* Perris (Col.: Curculionidae).** Ahmet Kati[1], Serpil Ugras[1,2], Huseyin Yilmaz[1,2], Hatice Kati[1]. [1]Giresun University, Giresun, TR; [2]Karadeniz Technical University, Trabzon , TR.

B-02 **The first study on the bacterial flora of the *Xyleborus xylographus* Say (Coleoptera: Curculionidae)**. Ahmet Kati[1], Serpil Ugras[1,2], Huseyin Yilmaz[1,2], Hatice Kati[1]. [1]Giresun University, Giresun, TR; [2]Karadeniz Teknik University, Trabzon, TR.

B-03 **Crystal structures of Cry34Ab1 and Cry35Ab1 proteins at 2.15 Å resolution**. Cheng Yang[1], Jim Pflugrath[1], Steve Evans[2] and Kenneth Narva[2], [1]Rigaku Americas, The Woodlands, TX, US; [2]Dow AgroSciences, Indianapolis, IN, US.

B-04 **Cysteine substitution in Cry7Ba1 crystal protein from *Bacillus thuringiensis* improves the crystal solubility and recovers its toxicity to *Plutella xylostella* larvae.** Donghai Peng, Fengshan Wang, Nisha Li, Zhengyu Zhang, Rong Song, Lifang Ruan , Ming Sun, Huazhong Agricultural University, Wuhan, CN.

B-05 **Screening and genetic characterization of *Bacillus thuringiensis* strains for the control of *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae).** F. P. Santos[1], J. Lopes[1], C. D. Carvalho-Filho[2], L. A. Vilas-Bôas[1], G. T. Vilas-Bôas[1], L. V. Leonel[1], F. A. P. Fazion[1], A.C. Dragalzew[1], J. A. Scarpassa[1], G. M. D. Souza[1], [1]Universidade Estadual de Londrina, Londrina/PR, BR; [2]Universidade Federal da Bahia, Salvador/BA, BR.

B-06 **Screening of *Bacillus thuringiensis* strains against economically important insect pests in Brazil**. G. T. Vilas-Bôas[1], P. M. O. J. Neves[1], L. F. A. Alves[2], F. Moscardi[1], R. G. Monnerat[3], D. R Sosa-Gómez[3], C. D. Carvalho-Filho[4], F. Cunha[5], K. C. C. Silva[1], T.M. Alexandre[5], [1]Universidade Estadual de Londrina, Londrina/PR, BR; [2] Universidade Estadual do Oeste do Paraná, Cascavel/PR, BR; [3]EMBRAPA, CENARGEN, Brasília/DF, BR; [4]Universidade Federal da Bahia, Salvador/BA, BR; [5]Universidade Federal do Paranandaacute;, Curitiba/PR, BR.

B-07 **Expression of Cry1AbMod toxin in transgenic tobacco and its effectiveness to control *Manduca sexta* larvae**. Helena Porta[1], Gladys Jiménez-Nopala[1], Elizabeth Cordoba[2], Mario Soberón[1], Bravo Alejandra[1], [1]Departamento de Microbiología, Cuernavaca, MX; [2]Departamento deBiologia Molecualr de Plantas, Cuernavaca, MX.

B-08 **Head-to-tail screening: An efficient approach to determine the activity–correlative regions of Cry proteins**. Jianqiao Zhou, Changlong Shu, Lili Geng, Zishan Zhou, Fuping Song, Dafang Huang, Jie Zhang, State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences (IPP CAAS), Beijing, CN.

B-09 **Evaluation of 2nd generation of *Bt*-transgenic sugar beet lines vs. their three lepidopteran pests**. Ladan Sedighi[1], Mohammadreza Rezapanah[2], Peyman Norouzi[3], [1]Islamic Azad University, Arak, IR; [2]Department of Biological Control, Iranian Research Institute of Plant Protection, Tehran, IR; [3]Sugar Beet Research Institute, Karadj, IR.

B-10 ***In vivo* virulence of *Bacillus cereus* and *Bacillus weihenstephanensis* at different temperatures in *Galleria mellonella* larvae**. Lotte Stenfors - Arnesen[1], Per Einar Granum[1], Christophe Buisson[2], Christina Nielsen-LeRoux[2], [1]Norwegian School of Veterinary Science, Oslo, NO; [2] INRA, Guyancourt, FR.

B-11 ***In vitro* assay of alternative phytosanitary products on *Bacillus thuringiensis* var. *kurstaki*.** Luis Francisco A. Alves[1], L. Martinelo[2], M.A Formentini[1], D. Thomazoni[1], L.P.C. Marchese[1], Vanda Pietrowski[1], [1]Western Paraná State University, Biotechnology Lab.Cascavel, PR, BR;

[2]Federal Center of Technological Education of Paraná, Dois Vizinhos, PR, BR.

B-12 **Biochemical and molecular characterization of delta-endotoxins in *Bacillus thuringiensis* native strain**. María E Vidal-Domínguez, Graciela L Salerno, Corina Berón, Centro de Estudios de Biodiversidad y Biotecnología-CIB Fundación para Investigaciones Biológicas Aplicadas (CEBB-CIB-FIBA-Mar del Plata) - CONICET, Mar del Plata, AR.

B-13 ***Yersinia entomophaga*, a potential new biopesticide for locusts**. Mark McNeill, Mark Hurst, AgResearch, Christchurch, NZ.

B-14 **Cloning, characterization and expression of *chitinase* A, B and C genes isolated from *Serratia marcescens* originating from *Helicoverpa armigera* and determining their insecticidal activity**. Mehtap Yakupoglu, Kazım Sezen, Ismail Demir, Zihni Demirbag, Remziye Nalcacıoglu, Karadeniz Technical University, Trabzon, TR.

B-15 **Effect of the symbiotic bacterium, *Xenorhabdus indica*, from S*teinernema abbasi* Taiwan strain on some microorganisms and insect cell lines.** Mi-Hau Tsai, Li-Cheng Tang, Roger F. Hou, Department of Entomology, National Chung Hsing University, Taichung, TW.

B-16 **Effect of *Bacillus thuringiensis* against *Lobesia botrana* Denis and Schiffermuller (Lepidoptera: Tortricidae) in Malekan Region**. Nouraddin Shayesteh[1], Mohammad Hassanzadeh[2], Mohammad Farshi Ghodsi[3], [1]Islamic Azad University, Barach of Mahabad, Mahabad, IR; [2]Urmia University, Agricultural Faculty, Department of Entomology, Urmia, IR; [3]Plant Protection Clinic of Malekan, Malekan, AZ.

B-17 **A cricket spiroplasma disease found in *Teleogryllus occipitalis* (Orthoptera: Gryllidae)**. Ping-Yi Su, Yu-Hsiang Hsu, Ching-Hao Chiang, Yu-Shin Nai, Chung-Hsiung Wang, Chu-Fang Lo, Institute of Zoology, National Taiwan University, Taipei, TW.

B-18 **Biodegradation of cypermethrin by a newly isolated Actinomycetes HU-S-01 from wastewater sludge.** Qingsheng Lin, Shaohua Chen, Meiying Hu, Liu Yang, Hui Li, Key Laboratory of Natural Pesticide and Chemical Biology, Ministry of Education, (South China Agricultural University), Guangzhou, CN.

B-19 **Histopathology of midgut in *Bacillus thuringiensis*- susceptible and resistant populations of Diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae).** Raziyeh Bakhshaei, Reza Talaei-Hassanloui, Reyhaneh Ezzati-Tabriz, Department of Plant Protection, University of Tehran, Karaj, IR.

B-20 **Natural infection of *Agriotes* sp. with *Rickettsiella* bacteria.** Regina G. Kleespies[1], Claudia Ritter[2], Andreas Leclerque[1], [1]Julius Kuhn-Institute, Institute for Biological Control, D-64287 Darmstadt,, DE; [2]Landesforschungsanstalt fur Landwirtschaft und Fischerei, D-18276 Gulzow, DE.

B-21 **Analysis of two toxic genes *xptA1* and *xptB1* in the entomopathogenic bacteria *Xenorhabdus nematophilus***. Ricardo Ferreira[1], Rafael Montiel[2], Nelson Simões[1], [1]CIRN, Ponta Delgada, PT; [2]CIRN, Irapuato, MX.

B-22 **Characterisation of an inhibitory compound (bacteriocin) from entomopathogenic *Bacillus thuringiensis* isolated from hazelnut beetle (*Balaninus nucum*.)** Serpil Ugras[1,2], Hatice Kati[2], Kazim Sezen[1], Zihni Demirbag[1], [1]Karadeniz Technical University, TR; [2]Giresun University, Giresun, TR.

B-23 **Genetic evidence of the protective role of the peritrophic matrix upon oral bacterial infection in *Drosophila melanogaster***. Takayuki Kuraishi, Bruno Lemaitre, EPFL, Lausanne, CH.

B-24 **Degenerate PCR based search for genes encoding insecticidal proteins of *Bacillus thuringiensis*.** Yu Karatani, Yoshinao Azuma, So Takebe, Kinki University, Kinokawa, Wakayama, JP.

B-25 **STU A study on the effect of sublethal doses of *Bacillus thuringiensis* on larvae of Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Col.: Chrysomelidae)**. Akbar Ghassemi Kahrizeh[1], Mohammad Hassan Saferalizadeh[2], Shahram Aramideh[2], Seyed Ali Safavi[2], Jamshid Akbarian[2], [1]Department of Plant Protection, Islamic Azad university, Branch of Mahabad, Urmia, IR; [2]Department of Plant Protection, College of Agriculture, University of Urmia, Urmia, IR.

B-26 **STU Investigation of interaction effects of *Bacillus thuringiensis* and azadirachtin on third larval stage of *Plodia interpunctella*.** Arman Abdolmaleki, Mohammad Hasan Safaralizadeh, Ali Safavi, Ramin Tandorost, Iman Sarifian, Mehdi Razmi, Gholamreza Sadeghi, Urmia University, Urmia, IR.

B-27 **STU *Bacillus thuringiensis* subsp. *kurstaki* (*Btk*) production and kinetics study in solid state fermentation with wheat bran and sugar cane bagasse**. Bruno Oishi, Adalberto Pessoa, Beatriz Kilikian, São Paulo University, São Paulo, BR.

B-28 **STU Study of the *Ostrinia nubilalis* Cry1Ab tolerance linkage to the *cadherin* locus**. Cristina M. Crava[1], Gema P. Farinos[2], Yolanda Bel[1], Pedro Castanera[2], Baltasar Escriche[1], [1]University of Valencia, Valencia, ES; [2]Centro de Investigaciones Biologicas - CSIC, Madrid, ES.

B-29 **STU Testing alkaline phosphatase from *Heliothis virescens* as functional Cry toxin receptor**. Jerreme Jackson[1], Omaththage P. Perera[2], Juan Luis Jurat-Fuentes[1], [1]University of Tennessee, Knoxville, US; [2]USDA-ARS Southern Insect Management Research Unit, Stoneville, US.

B-30 **STU Head-to-tail screening: An efficient approach to determine the activity–correlative regions of Cry proteins.** Jianqiao Zhou[1], Changlong Shu[1], Lili Geng[1], Zishan Zhou[1], Fuping Song[1], Dafang Huang[2], Jie Zhang[1], [1]State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences(IPP CAAS), Beijing, CN: [2]Plant Protection, Chinese Academy of Agricultural Sciences(IPP CAAS), Beijing, CN.

B-31 **STU The repeat region within Orf2 protein is a significant factor in Cry2Aa toxin crystallization.** Junlan Ma[1], Neil Crickmore[2], Changlong Shu[1], Lin Zhou[1], Jie Zhang[1], Fuping Song[1], Dafang Huang[3], [1]State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, CN; [2]School of Biological Sciences, University of Sussex, Brighton, GB; [3] Biotechnology Research InstituteChinese Academy of Agricultural Sciences, Beijing, CN.

B-32 **STU Activation process of the coleopterancidal Cry8D on leaf-beetles.** Keika Yamada, Takuya Yamaguchi, Ken Sahara, Hisanori Bando, Shin-ichiro Asano, Department of Applied Bioscience,

Graduate School of Agriculture, Hokkaido University, N9W9 Sapporo Hokkaido, JP.

B-33 **STU The first study on bacterial flora and biological control agent of fruit trees pest beetles *Sciaphobus squalidus* (Gyll.), *Tatianaerhynchites aequatus* (L.) and *Byctiscus betulae* L.in Republic of Moldova**. Natalia Munteanu[1], Mehtap Yakupoglu[2], Ion Toderas[1], Remziye Nalcacioglu[1], Zihni Demirbag[1]. [1]Centre of General and Molecular Biology, Institute of Zoology, Moldova Academy of Science, Chisinau, MD; [2]Karadeniz Technical University, Trabzon, TR.

B-34 **Characterization and pathogenic evaluation of *Bacillus thuringiensis* isolates from west Azerbaijan province-Iran**. Shahram Aramideh, Mohammad Hassan Saferalizadeh, Ali Asghar Pourmirza, Mahmood Rezazadeh Bari, Mansureh Keshavarzi, Mahdi Mohseniazar, Urmia, IR

B-35 **STU Isolation and diversity of *Bacillus thuringiensis* and insecticidal activity against red flour beetles (*Tribolium castaneum*)**. Shahram Aramideh, Mohammad Hassan Saferalizadeh, Ali Asghar Pourmirza, Mahmood Rezazadeh Bari, Mansureh Keshavarzi, Mahdi Mohseniazar. Urmia, IR.

B-36 **Isolation and identification native *B. thuringiensis* in different habitat from west Azerbaijan and evaluate effects on Indian moth *(Plodia interpunctella*)**. Shahram Aramideh, Mohammad Hassan Saferalizadeh, Ali Asghar Pourmirza, Mahmood Rezazadeh Bari, Maansureh Keshavarzi, Mahdi Mohseniazar, Urmia, IR

B-37 **STU Intramolecular proteolytic nicking and binding of *Bacillus thuringiensis* Cry8Da toxin in BBMVs of Japanese beetle.** Takuya Yamaguchi, Ken Sahara, Hisanori Banado, Shin-ichiro Asano, Department of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Sapporo, JP.

COST 862

BC-01 **Occurrence of carabid beetles in *Bt*-maize fields**. Adriana Simanska, Peter Bokor, Ludovit Cagan, Slovak Agricultural University in Nitra, Nitra, SK.

BC-02 **An alternative set of test to bioassay for bioinsecticides**. Barbara Manachini[1], Mirella Vazzana[1], Sergio Franceschini[2], Vincenzo Arizza[1], [1]Dept. Animal Biology University of Palermo, Palermo, IT; [2]Intrachem Production Grassobbio, Italy, Bergamo, IT.

BC-03 **Proteolytic processing of *Bacillus thuringiensis* Cry3Aa toxin is a key step in toxicity against Colorado potato beetle**. Camila Ochoa-Campuzano, Jorge Sánchez, Inmaculada García-Robles, M. Dolores Real, Carolina Rausell, Universidad de Valencia, Valencia, ES.

BC-04 **Susceptibility to *Bt* of wild lepidopteran species in nature reserve in Sicily**. Filippo Castiglia[1], Barbara Manachini[2], [1] Azienda Foreste Demaniali Regione Sicila, Palemro, IT; [2]Dipartimento Biologia Animale Università degli Studi di Palermo, Palermo, IT.

BC-05 **Activity of Cyt1Aa protein from *Bacillus thuringiensis* subsp. *israelensis* against the Mediterranean fruit fly, *Ceratitis capitata*.** Joel González-Cabrera, José Cristian Vidal-Quist, Moncada, Valencia, Entomology Asociate Unit (IVIA-UJI-CIB). IVIA-Crop Protection Department, Moncada, Valencia, ES.

BC-06 **Susceptibility of the 4th instar larvae of *Spodoptera exigua* (Hubner) (Lepdoptera: Noctuidae) to VIP3Aa toxin**. Juliana-Regina Vieira-Costa[1], Patricia Hernández-Martínez[2], Juan Ferré[1], Baltasar Escriche[1], [1]Department of Genetics, University of Valencia, València, ES; [2]Department of Environmental Biology, Centro de Investigaciones Biológicas, CSIC, València, ES.

BC-07 **Influence of *Bt* maize hybrids to predatory insects.** Kristina Stanikova, Ludovit Cagan, Slovak Agricultural University in Nitra, Nitra, SK.

BC-08 ***In vivo* modulation of Hsp70 in *Rhynchophorus ferrugineus* hemocytes after *Bacillus thuringiensis* treatment.** Monica Celi, Mirella Vazzana, Barbara Manachini, Nicolò Parrinello, Vincenzo Arizz, Dept. Animal Biology University of Palermo, Palermo, IT.

BC-09 **Effects of Cry3Bb1-maize on the two-spotted spider mite**. Rostislav Zemek, Oxana Habustova, Hany M. Hussein, Zdenka Svobodova, Frantisek Sehnal, Institute of Entomology, BC AS CR, Ceske Budejovice, CZ.

BC-10 **Carboxy-terminal extension effects on crystal formation and insecticidal properties of Cry15Aa**. Samir Naimov[1], Rumyana Valkova1, Ruud deMaagd[2], [1]University of Plovdiv "Paisii Hilendarski", 4000 Plovdiv, Bulgaria, Plovdiv, BG; [2]Plant Research International, Business Unit Bioscience, 6700 AP Wageningen, NL.

BC-11 **Molecular characterization of putative receptors of the Bt toxin Cry3A in *Chrysomela tremulae***. Manuella Van Munster[1], Marie Le Gleuher[1], Yannick Pauchet[2], Marcel Amichot[1], Richard ffrench-Constant[2], David Pauron[1], [1]INRA, Sophia Antipolis, FR; [2]University of Exeter, Penryn, GB.

BC-12 **Effect of *Bacillus thuringiensis* on respiration rates of marine intertidal *Mytilaster intertidal, Mytilaster minimus* (Mollusca, Bivalvia)**. Vincenzo Arizza[1], Barbara Manachini[1], Gianluca Sarà[2], [1]Dipartimento di Biologia Animale "G. Reverberi", Università di Palermo, Palermo, IT; [2] Dipartimento di Ecologia, Università di Palermo, Palermo, IT.

BC-13 **Effectiveness and host range of endotoxin producing wild isolates of *Bacillus thuringiensis***. Zane Metla, Julija Halimona, Rita Seskena, Valentina Petrova, Liga Jankevica, Institute of Biology, University of Latvia, Salaspils, LV.

BC-14 **Midgut microflora of sawfly, pine looper and gypsy moth with emphasis on *Bacillus* genus bacteria.** Zane Metla, Julija Halimona, Rita Seskena, Valentina Petrova, Liga Jankevica, Institute of Biology, University of Latvia, Salaspils, LV.

BC-15 **Resistance of *Plutella xylostella* to *Bt* and synthetic insecticides.** Ali Sayyed[1], Sahar Fazal[1], Balu Venkatasamy[1], Asim Gulzar[2], Denis Wright[2], Neil Crickmore[1], [1]School of Life Sciences, University of Sussex, Brighton, GB; [2]Imperial College, London, GB.

BC-16 **STU Expression of *Bt* toxins and their hybrids, and their toxicity towards *Plutella xylostella.*** Zenas George[1], Changlong Shu[2], Jie Zhang[2], Neil Crickmore[1], [1]School of Life Sciences, University of Sussex, Brighton, GB; [2]Chinese Academy of Agricultural Sciences, Beijing, CN.

VIRUSES

V-01 **Effect of host plant on the persistence of nucleopolyhedrovirus isolates of *Helicoverpa armigera* (HearNPV) under open weather conditions.** Ali Mehrvar Mehrvar, Academic staff of the University of Maragheh, Maragheh, IR.

V-02 **Natural occurrence of *Helicoverpa armigera* Nucleopolyhedrovirus and a fast screening of isolates derivated from tobacco farms of north of Iran**. Hoda Assemi[1], Mohammadreza Rezapanah[2], [1]Islamic Azad University, Arak, IR; [2] Insect virology lab, Biocontrol Dept, Iranian Research Institute of Plant Protection, Tehran, IR.

V-03 **Possibilities of using baculovirus product Madex ® for control of *Cydia pomonella* (L.), in Bulgaria**. Hristina Kutinkova[1], Vasiliy Dzhuvinov[1], Jörg Samietz[2], Daniel Zingg[3], Philip Kessler[3], [1]Fruit Growing Institute, Plovdiv, BG; [2]Swiss Federal Research Station Agroscope Changins-Wädenswil ACW, Wädenswil , CH; [3]Andermatt Biocontrol AG, CH-6146 Grossdietwil, CH.

V-04 **Presence of nucleopolyhedrovirus in populations of the gypsy moth *Lymantria dispar* L. (Lepidoptera: Lymantriidae) in Latvia**. Liga Jankevica[1], Julija Halimona[1], Zane Metla[1], Rita Seskena[1], Regina G. Kleespies[2], Ivars Zarins[1], [1]Department of Experimental Entomology and Biology, Institute of Biology, University of Latvia, Salaspils, LV; [2] Julius Kuhn-Institute (JKI), Federal Research Centre for Cultivated Plants, Institute for Biological Control, Darmstadt, DE.

V-05 **Spread of a nucleopolyhedrovirus within populations of balsam fir sawfly (*Neodiprion abietis*) larvae following its aerial application.** Roger Graves[1], Dan Quiring[1], Christopher Lucarotti[2], [1]Faculty of Forestry and Environmental Management, University of New Brunswick, Fredericton, CA; [2]Canadian Forest Service – Atlantic, Fredericton, CA.

V-06 **Transmission of a nucleopolyhedrovirus within cohorts of balsam fir sawfly *(Neodiprion abietis*) larvae**. Roger Graves[1], Dan Quiring[1], Christopher Lucarotti[2]. [1]Faculty of Forestry and Environmental Management, University of New Brunswick, Fredericton, CA; [2]Canadian Forest Service – Atlantic, Fredericton, CA

V-07 **Physical characteristics of Oryctes virus that influence infectivity.** Sean Marshall, Craig Bunt, Trevor Jackson, AgResearch, Lincoln, NZ.

V-08 **STU Characteristic genomic features of the fast-killing *Epinotia aporema* Granulovirus**. María Leticia Ferrelli[1], Ricardo Salvador[1], Marcelo Facundo Berretta[2], Pablo Daniel Ghiringhelli[3], Alicia Sciocco-Cap[2], Víctor Romanowski[1], [1]IBBM-UNLP-CONICET, La Plata, AR; [2]IMyZA-INTA, Castelar, AR; [3]LIGBCM-UNQ, Quilmes, AR.

V-09 **STU Development of a PCR based method for identification, discrimination and quantification of baculoviruses specific for cutworms, *Agrotis* sp.** Jörg T. Wennmann[1], Wael El-Menofy[2], Waly Essam[2] , Naglaa Abdallah[2], Johannes A. Jehle[1], [1]Institute for Biological Control, Julius Kuhn-Institute, Federal Research Centre for Cultivated Plants, Darmstadt, DE; [2]Faculty of Agriculture, Cairo University, Giza, EG.

V-10 **Novel picorna-like virus from *Spodoptera exigua***. Ana Isabel Millan Leiva, Agata K. Jakubowska, Juan Ferre, Salvador Herrero. Department of Genetics, University of Valencia, Burjassot, ES.

V-11 **Analysis of Wuhan nodavirus genomic structure and characterization of its nonstructural protein B2**. Jiamin Zhang, Dawei Cai, Congyi Zheng, Yuanyang Hu, Wuhan University, Wuhan, CN.

V-12 **Analysis of type 5 *Helicoverpa armigera* cypovirus genome**. Yuanyang Hu, Li Tan, Congyi Zheng , Jiamin Zhang, Wuhan University, Wuhan, CN.

V-13 **Sequence analysis on the genome of the *Choristoneura biennis* entomopoxvirus**. Zhen Li[1], Daniel Doucet[2], Peter Krell[3], Basil Arif[1], [1]Laboratory for Molecular Virology, GLFC, Sault Ste Marie, ON, CA; [2]GLFC, Sault Ste Marie, ON CA; [3]Molecular and Cellular Biology, Guelph, ON, CA.

V-14 **STU Sequence and genomic analysis of the *Mamestra brassicae* nucleopolyhedrovirus-K1 isolated from Korea**. Jae Bang Choi, Jae Kyung Lee, Tae Young Shin, Sung Min Bae, Hyun Na Koo, Soo Dong Woo, Chungbuk National University, Cheongju, KR .

V-15 **AcMNPV LEF-2 is a capsid protein required for amplification but not initiation of viral DNA replication.** Carol P. Wu, Yi-ju Huang, Jen-yeu Wang, Yu-chan Chao, Institute of Molecular Biology, Taipei, TW.

V-16 **Expression and analysis of the baculovirus P10 protein in mammalian cells**. Farheen Raza, Caroline Griffiths, John Runions, Linda King Oxford Bookes University, Oxford, GB.

V-17 **Stability analysis of many polyhedra variants of *Anticarsia gemmatalis* MNPV baculovirus**. Juliana Carvalho Rangel, William Sihler, Maria Elita Batista de Castro, Marlinda Lobo de Souza, Embrapa Recursos Geneticos e Biotecnologia, Brasilia, BR.

V-18 **Identification and preliminary characterization of a chitinase gene in the *Epinotia aporema* granulovirus genome**. Ricardo Salvador[1], Leticia Ferrelli[2], Marcelo Berretta[1], Víctor Romanowski[2], Alicia Sciocco-Cap[1], [1]IMYZA-INTA; IBBM-UNLP, Buenos Aires, AR; [2]IBBM-UNLP; CONICET, La Plata, AR.

V-19 **Early gene hhi1 of HzNV-1 virus is a strong apoptosis inducer and crucial for latent viral re-activation**. Yueh-Lung Wu, Yu-Chan Chao**,** Taipei, TW.

V-20 **Chikungunya virus nonstructural protein 2 is a potent inhibitor of JAK-STAT signaling.** Jelke Fros, Corinne Geertsema, Maarten Ligtenberg, Esther Schnettler, Just Vlak, Gorben Pijlman, Laboratory of Virology, Wageningen University, Droevendaalsesteeg 1, 6708 PB , Wageningen, NL.

V-21 **STU Analysis of baculovirus gene function in insect cells**. Adam C. Chambers[1], Robert D. Possee[2], Richard B. Hitchman[1], Linda A. King[1], [1]Oxford Brookes University, School of Life Sciences, Oxford, GB; [2]CEH Oxford, Oxford, GB;- Oxford Brookes University, School of Life Sciences, Oxford, GB.

V-22 **STU Essential genes of BmNPV**. Chikako Ono, Ken Sahara, Shin-ichiro Asano, Hisanori Bando, Graduate School of Agriculture, Hokkaido University, Sapporo, JP.

V-23 **STU hr5 is a shut-off escaping element in the baculovirus-infected cells**. Daisuke Ohtsuka, Shin-ichiro Asano, Ken Sahara, Hisanori Bando Laboratory of Applied Molecular Entomology, Hokkaido University, Sapporo, JP.

V-24 **STU Molecular analysis of ORF AMV133 encoded by *Amsacta moorei* Entomopoxvirus (AmEPV)**. Emine Demir, Remziye Nalcacioglu, Zihni Demirbag. Karadeniz Technical University, Trabzon, TR.

V-25 **STU The AcMNPV ptp gene induces hypermobile behavior in *Spodoptera exigua* larvae**. Stineke van Houte[1], Kelli Hoover [2,] Just M. Vlak[1], Monique M. van Oers[1], [1]Laboratory of Virology, Wageningen University, Droevendaalsesteeg 1, 6708 PB, Wageningen, NL; [2]Laboratory of Entomology, Pennsylvania State University, 501 ASI, University Park, PA 16802, US.

V-26 **A new baculovirus vector for expression of foreign genes in the *Lymantria xylina* larvae and cell lines.** Chih-Yu Wu, Chung-Hsiung Wang, Chu-Fang Lo, Institute of Zoology, National Taiwan University, Taipei, Taiwan, R.O.C, Taipei, TW.

V-27 **STU Construction of recombinant baculoviruses without using cell cultures**. Kaoru Teduka, Yasuhisa Kunimi, Ayako Hirao, Madoka Nakai, Tokyo University of Agriculture and Technology, Tokyo, JP.

V-28 **STU Enhanced production of CSFV E2 protein by fusion expression with partial polyhedrin of nucleopolyhedrovirus.** Sung Min Bae[1], Hyun Na Koo[1], Bit Na Rae Yun[1], Tae Young Shin[1], Jae Bang Chou[1], Soo Dong Woo[1], Yeon Ho Je[2], Byung Rae Jin[1], [1]Department of Agricultural Biology, Chungbuk National University, Cheongju, KR; [2]School of Agricultural Biotechnology, College of Agriculture and Life Sciences, Seoul National University, Seoul, KR

V-29 **Baculovirus-expressed haemagglutinin (HA) of an influenza H5N1 virus as a diagnostic reagent.** David Leishman, Dan-Hui Yang, Yanlong Pei, Peter Krell, Eva Nagy, University of Guelph, Guelph, CA.

V-30 **Chikungunya virus glycoprotein expression by recombinant baculoviruses in insect cells**. Stefan Metz, Corinne Geertsema, Just Vlak , Gorben Pijlman, Laboratory of Virology, Wageningen University, Droevendaalsesteeg 1, 6708 PB, Wageningen, NL.

V-31 **STU Cloning and expression of the glycoproteins gB, gC, and gD of Aujeszky's Disease Virus NYJ strain in *Bombyx mori* cells and larva.** Hyun Na Koo, Bit Na Rae Yun, Sung Min Bae, Tae Young Shin, Jae Bang Choi, Soo Dong Woo, Jae Young Choi, Kwang Sik Lee, Jong Yul Roh, Yeon Ho Je Byung Rae Jin. Department of Agricultural Biology, Chungbuk National University, Cheongju, KR.

V-32 **STU Deacetylation of GD3A using baculovirus-a novel therapeutic approach for invasive glioma.** John Owsus Danquah[1], Suzanne Birks[2], Richard Hitchman[3], Ananya Jeshtadi[1], Reinhard Vlasak[4], Darek Gorecki[2], Linda A King[1], Geoffrey John Pilkington[2], [1]Oxford Brookes University, Oxford, GB; [2]University of Portsmouth, Portsmouth, GB; [3]Oxford Expression Technologies Ltd, Oxford, GB; [4]University of Salzburg, Salzburg, AT.

V-33 **A deletion virus for improved recombinant protein expression**. Richard Hitchman, Robert D. Possee, Andrew T. Crombie, Adam Chambers, Kim Ho, Evangelia Siaterli, Olga Lissina, Heather Sternard, Robert Novy, Kathryn Loomis, Louise E. Bird, Raymond J. Owens , Linda A. King, Oet ltd, Oxford, GB.

V-34 **Identification of proteins associated with *Helicoverpa armigera* nucleopolyhedrovirus budded virions**. Dianhai Hou[1], Fei Deng[1,] Hualin Wang[1], Leike Zhang[2], Lin Guo[2], Zhihong Hu[1], [1]Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, CN; [2] Wuhan University and College of Life Sciences, Wuhan, CN.

V-35 **Functional analysis of N-linked glycosylation of *Helicoverpa armigera* single nucleocapsid nucleopolyhedrovirus envelope fusion protein F**. Shu Shen[1], Manli Wang[1], Qing Lan[1], Shufen Li[1], Xin L[i1], Hualin Wang[1], Just M. Vlak[2], Fei Deng[1], Zhihong Hu[1], [1]Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, CN; [2]Laboratory of Virology, Wageningen University, Wageningen, NL.

FUNGI

F-01 **Effects of certain pre-incubation and incubation conditions on the zygospore germination of *Conidiobolus osmodes* Drechsler (Arcylistaceae: Entomophthorales).** Abdurrahman Adin[1], Mehmet Kubilay Er[2]. [1]Tavsanlı Tarım Ilce Mudurlugu, Tavsanlı, Kutahya, TR; [2]Department of Plant Protection, Faculty of Agriculture, University of Kahramanmaras Sutcu Imam, Kahramanmaras, TR.

F-02 **Extraction and characterization of extracellular protease from the entomopathogenic fungus, *Beauveria bassiana* in the presence of *Eurygaster integriceps* (Hemiptera: Scutelleridae) cuticle**. Ali Reza Bandani, Plant Protection Department, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran., Karaj, IR.

F-03 **Fungi related to larvae of red palm weevil *Rhynchophorous ferrugineus* (Coleoptera: Curculionidae)**. Antonietta Vella[1], Barbara Manachini[2], Franco Palla[1], [1]Dipartimento di Scienze Botaniche Università di Palermo, Palermo, IT; [2]Dipartimento di Biologia Animale "G. Reverberi", Università di Palermo, , Palermo, IT.

F-04 **Combination of entomopathogenic fungi *Beauveria bassiana* (Bals.) and *Lecanicillium muscarium* (Petch.) with insecticide imidacloprid on different nymphal instars of greenhouse whitefly *Trialeurodes vaporariorum* West. (Hemiptera: Aleyrodidae) in laboratory conditions**. Bijan Hatami[1], Naser Malekan[2], Rahim Ebadi[2], Alireza Akhavan[2], [1]Islamic Azad University of Khorasgan, Isfahan, IR; [2]Isfahan University of Technology, Isfahan, IR.

F-05 **The effect of selected plant volatiles on conidial germination of aphid-pathogenic fungi**. Cezary Tkaczuk[1,] Robert Krzyżanowski[2], Bogumił Leszczyński[2,] Tomasz Krzyczkowski[1], Bernard Papierok[3], [1]Department of Plant Protection, University of Podlasie, Siedlce, PL; [2]Department of Biochemistry and Molecular Biology, University of Podlasie, Siedlce, PL; [3]Collection des Champignons, Institute Pasteur, Paris, FR.

F-06 **Horizontal transmission of *Lecanicillium* spp. from infected cadaver of cotton aphid to healthy population**. Daigo Aiuchi[1], Toshihiro Watanabe[2], Masanori Koike[2], [1]National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080-8555, Japan, Obihiro, JP; [2]Department of Agro-environmental Science, Obihiro University of

Agriculture and Veterinary Medicine, Obihiro 080-8555, Japan, Obihiro, JP.

F-07 **Virulence of the entomopathogenic fungi isolated from the great spruce bark beetle, *Dendroctonus micans* (Kugelann) (Coleoptera: Scolytidae)**. Elif Tanyeli[1], Ali Sevim[2], Zihni Demirbag[1], Mahmut Eroglu[1] and Ismail Demir[1], [1]Karadeniz Technical University, Trabzon, TR; [2]Karadeniz Technical University, Rize University, Rize, TR.

F-08 **CTC medium: A novel dodine-free selective medium for isolating entomopathogenic fungi, especially *Metarhizium acridum*, from soil**. Éverton Fernandes[1], Chad Keyser[1], Drauzio Rangel[2], Donald Roberts[1], [1]Utah State University, Logan, Utah, US; [2]Universidade do Vale do Paraíba, São Jose dos Campos, SP, BR.

F-09 **Both alpha and beta tubulins are involved in *Metarhizium anisopliae* resistance to carbendazim**. Gang Zhou, Sheng-hua Ying, Ming-guang Feng, Zhejiang University, Hangzhou, CN.

F-10 **Influence of host plant species on Diamondback moth, *Plutella xylostella* susceptibility to *Beauveria bassiana* and *Metarhizium anisopliae* and on its fertility life table parameters**. Hosna Mohammadi-Tabar, Reza Talaei-Hassanloui, Mohammad Mehrabadi , Hossein Allahyari. Department of Plant Protection, University of Tehran, Karaj, IR.

F-11 **Molecular characterization and virulence of *Beauveria* spp. from the pine processionary moth, *Thaumetopoea pityocampa* (Lepidoptera: *Thaumetopoeidae*)**. Ismail Demir[1], Ali Sevim[2], Zihni Demirbag[1], [1]Karadeniz Technical University, Trabzon, TR; [2]Rize University, Rize, TR.

F-12 **New insights into biocontrol of the white grub, *Polyphylla olivieri***. Javad Karimi[1], Aziz Kharazi-Pakdel[2], [1]Department of Plant Protection, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, IR; [2]Campus of Agricaulture and Natural resources, University of Tehran, Karaj, IR.

F-13 **First evaluation of *Beauveria bassiana* against the *Gyropsylla spegazziniana* lizer and trelles (Hemiptera: Psilidae) on Paraguay tea (*Ilex paraguariensis* St. Hil.) leaves.** Luis Francisco A. Alves[1], A.L.P. Fanti[1], M.A. Formentini[1], I.L.M. Barzotto[1], M.E. Formentini Schapovaloff[2], [1]Western Paraná State University, Biotechnology Lab., Cascavel, PR, BR; [2]National University of La Plata, AR.

F-14 ***Evlachovaea*: Coming home to say goodbye!** Luiz F.N. Rocha[1], Peter W. Inglis[2], Richard A. Humber[3], Andre Kipnis[1], Christian Luz[1], [1]IPTSP, Universidade Federal de Goias, Goiania, BR; [2]Embrapa Cenargen, Brasilia, BR; [3]USDA-ARS BioIPM Research, RW Holley Center for Agriculture and Health, Tower Road, Ithaca, New York, US.

F-15 **Longevity and fertility life table parameters of *Phytoseiulus persimilis* (Acari: Phytoseiidae) fed on untreated and *Beauveria bassiana* treated adults of *Tetranychus urticae* (Acari: Tetranychidae)**. Marjan Seiedy, Alireza Saboori, Hossein Allahyari, Reza Talaei-Hassanloui, Mahdi Tork, University of Tehran, Karaj, IR.

F16 **Preliminary results on the occurence of pathogens in the pine shoot beetles *Tomicus piniperda* (L) (Coleoptera, Scolytidae) in Georgia**. Medea Burjanadze, Archil Supatashvili, Vasil Gulisashvili Forest Institute, Tbilisi, GE.

F-17 **Evaluation of *Isaria fumosorosea* CCM 8367 for the control of *Cameraria ohridella*, and effects on beneficial parasitoids**. Mona Awad[1], Eva Prenerova[2], Lubomir Volter[1], Rostislav Zemek[1]. [1]Institute of Entomology, BC AS CR, Ceske Budejovice, CZ; [2]Laboratory of Plant Protection, Olesna, CZ.

F-18 **Entomopathogenic fungus of *Coccinella septempunctata* L. (Col.: Coccinellidae) in the Uludaz Hill of the Cimen Mountain, Kahramanmaras**. Ozlem Kalkar[1], Engin Kılıc[2], [1]Kahramanmaras Sutcu Imam University, Kahramanmaras, TR; [2]Erzincan University Uzumlu MYO, Erzincan, TR.

F-19 **Study of entomopathogenic fungi *Aschersonia* isolates from Chinese citrus orchards on pathogenicity, phylogeny and genetic diversity.** Ping-ping Wang, Hong-yu Zhang, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan, CN.

F-20 **The melanin produced by recombinant *Escherichia coli* enhances the survival of *Beauveria bassiana* conidia under UV-B irradiation**. Sheng-hua Ying, Ming-guang Feng, Zhejiang University, Hangzhou, CN.

F-21 **Pathogenicity of selected entomopathogenic fungi to larvae of *Spodoptera littoralis* (Boisd.) (Lep.: Noctuidae) in laboratory conditions**. Soner Cerci[1], Mehmet Kubilay Er[2], [1]Turkoglu Ilce Tarim Mudurlugu, Turkoglu, Kahramanmaras, TR; [2]Department of Plant Protection, Faculty of Agriculture, University of Kahramanmaras Sutcu Imam, Kahramanmaras, TR.

F-22 **Endophytic colonization of entomopathogenic fungi in strawberry plants**. Surendra Dara[1], Sudha Dara[2], [1]University of California Cooperative Extension, Santa Maria, US; [2]Cachuma Resource Conservation District, Santa Maria, US.

F-23 **Perspectives of the Colorado potato beetle fungi pathology in Georgia.** Tsisia Chkhubianishvili[1], Manana Kakhadze[1], Iatamze Malania[1], Mark Goettel[2], [1]Kanchaveli L. Institute of Plant Protection, Tbilisi, GE; [2]Lethbridge Research Centre, Agriculture and Agri-Food Canada, Lethbridge, CA.

F-24 **Characterization of three different catalase genes in entomopathogenic fungus *Beauveria bassiana***. Zheng-liang Wang, Sheng-hua Ying, Ming-guang Feng, Zhejiang University, Hangzhou, CN.

F-25 **Functional characterization of hydrophobins in the entomopathogeneic fungus *Metarhizium anisopliae***. Ali Sevim[1], Dongliang Wu[2], Zihni Demirbag[3], B.Gillian Turgeon[2], [1]Rize University, Rize, TR; [2] Department of Plant Pathology and Plant-Microbe Biology, 344 Plant Science Building, Cornell University, New York, US; [3]Karadeniz Technical University, Trabzon, TR.

F-26 **STU Susceptibility of *Ceratitis capitata* to *Beauveria bassiana* isolates from the Moroccan endemic forests of *Argania spinosa***. Imoulan Abdessamad A. Imoulan, El Meziane Abdellatif, Laboratory of Biotechnology, Valorisation and Protection of Agro-Resources, FST, Cadi-Ayyad University, B.P. 549 , Marrakesh, MA.

F-27 **STU Isolation of anamorphic entomopathogenic fungi from wild mosquitoes collected in Japan and Burkina-Faso**. Mitsugu Ishiyama[1], Masanori Koike[1], Shinya Fukumoto[2], Hirotaka Kanuka[2], Junya Takeshita[1], Daigo Aiuchi[2], [1]Department of Agro-environmental Science, Obihiro University of

Agriculture and Veterinary Medicine, Obihiro, JP; [2]National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, JP.

F-28 **STU Occurrence and genetic diversity of native entomopathogenic fungi from soils in Korea**. Tae Young Shin, Sung Min Bae, Jae Bang Choi, Bit Na Rae Yun, Hyun Na Koo, Soo Dong Woo. Chungbuk National University, Cheongju, KR.

F-29 **STU Pathogenicity of *Beauveria bassiana* and *Metarhizium anisopliae* to the *Tetranychus urticae* and *Metaseiulus occidentalis*.** Tea Abramishvili[1], Medea Burjanadze[2], [1]Georgian State Agrarian University, Tbilisi, GE; [2]Vasil Gulisashvili Forest Institute, Tbilisi, GE.

F-30 **STU Interaction between *Lecanicillium* spp. and *Aphidius colemani* in biological control for *Aphis gossipii***. Yuuna Saitou[1], Junya Tone[1], Daigo Aiuchi [2], Masanori Koike[1], [1]Department of Agro-environmental Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, JP; [2]National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, JP.

F-31 ***Paecilomyces lilacinus* – from nematophagous fungus in field conditions to entomopathogenic fungus in greenhouse conditions.** Danuta Sosnowska, Zaneta Fiedler, Institute of Plant Protection – National Research Institute, Wladyslawa Wegorka 20 str., 60-318 Poznan, Poland

Thursday July 15, 2010

Osman Turan Congress Center

07:30 Bus pick up at hotels

Cross Divisional Symposium

Thursday, 8:00-10:00
Hasan Turan

Nematode and Fungus Divisions

Formulation of Entomopathogenic Fungi and Nematodes to Overcome Environmental Limitations

Organizer: Lawrence Lacey

8:00 **182 Formulation and application of *Metarhizium acridum* for control of locusts: overcoming the limitations of dry environments**. Roy Bateman[1], Christiaan Kooyman[2], [1]IPARC, c/o Imperial College London, Ascot, GB; [2]Fondation Agir pour l'Education et la Santé, Dakar, SN.

8:30 **183 Novel approaches in formulation of entomopathogenic fungi for control of insects in soil, foliar, and structural habitats: Thinking outside the box and expecting the unexpected**. Stefan Jaronski[1], Mark Jackson[2], Christopher Dunlap[2], William Meikle[3], [1]USDA ARS, Sidney MT, US; [2]USDA ARS, Peoria IL, US; [3]USDA ARS, Weslaco TX, US.

9:00 **184 Formulation and application: key technologies to expand the use of entomopathogenic nematodes**.
Arne Peters, e-nema GmbH, Schwentinental, DE.

9:30 **185 Formulation and environmental manipulation to enhance the larvicidal activity of entomopathogenic nematodes for control of insect pests of orchards**. Lawrence Lacey , David Shapiro-Ilan, USDA-ARS, Wapato, WA, US.

Contributed Papers

Thursday, 8:00-10:15
Fahri Kuran

BACTERIA 4

Chairs: Baltasar Escriche and Neil Crickmore

8:00 **186-STU Novel members of the repat genefamily and their expression in the midgut of *Spodoptera exigua* larvae in response to pathogens**. Gloria Navarro-Cerrillo, Manuela Barneo-Muñoz, Juan Ferré, Salvador Herrero, University of Valencia, Burjassot, ES.

8:15 **187 Screening of *Bacillus thuringiensis* isolates against *Agrotis segetum* with specific focus on vegetative insecticidal proteins**. Dorra Ben Hamadou[1], Annette Sauer[1], Samir Jaoua[2], Dietrich Stephan[1], [1]nn, Darmstadt, DE; [2]nn, Qatar, QA.

8:30 **188 Proteolytic processing of *Bacillus thuringiensis* Cry7Aa1 toxin and specific binding to brush border membrane vesicles of three sweetpotato weevil species (Coleoptera: Brentidae)**. Patricia Hernández-Martánez[1], Willian Moar[2], Baltasar Escriche[1], [1]Department of Genetics, Universitat de València, Burjassot, ES; [2]Department of Entomology and Plant Pathology, Auburn University, Auburn, US.

8:45 **189 Analysis of the effect of mutations in domain II of *Bacillus thuringiensis* Cry3Aa toxin**. Inmaculada García-Robles, Camila Ochoa-Campuzano, Amparo Consuelo Martínez-Ramírez, Carolina Rausell, M. Dolores Real, University of Valencia, Burjassot Valencia, ES.

9:00 **190 Gene dosage analysis in the European corn borer *Ostrinia nubilalis* (Lepidoptera) using quantitative real-time PCR with SYBR green detection**. Yolanda Bel, Juan Ferre, Baltasar Escriche, Universitat de Valencia, Burjassot, ES.

9:15 **191 Restoration of the crystallisation of altered delta-endotoxins Cry1Ac, by the promotion of their *in vivo* integration into the *Bacillus thuringiensis* native crystal**. Tounsi Slim, Dammak Mariam, Jaoua Samir, Centre of biotechnology of Sfax, Sfax, TN.

9:30 **192-STU Role of Cry3Aa domain II loop 1 in the mode of action of Cry3Aa toxin**. Fernando Zúñiga, Isabel Gómez, Ernesto Ortiz, Alejandra Bravo, Mario Soberón, Instituto de Biotecnología, UNAM, Cuernavaca, MX.

9:45 **193 Identification of key regulators controlling expression of the fit insect toxin in the root-associated biocontrol pseudomonad CHA0**. Vincent Turner[1], Maria Péchy-Tarr[1], Naomi Borel[1], Olivier Binggeli[1], Monika Maurhofer[2], Christoph Keel[1], [1]Department of Fundamental Microbiology, University of Lausanne, Lausanne, CH; [2]Plant Pathology, Institute of Integrative Biology, ETH Zurich, Zurich, CH.

10:00 **194 Biochemical basis of field-isolated resistance to *Bacillus thuringiensis* Cry2A insecticidal proteins in *Helicoverpa* species.** Silvia Caccia[1], Carmen Sara Hernández-Rodríguez[1], Rod J. Mahon[2], Sharon Downes[3], William James[2], Nadine Bautsoens[4], Jeroen Van Rie[4] and Juan Ferré[1] , [1]Department of Genetics, University of Valencia, Dr Moliner 50, 46100-Burjassot, Spain, [2]CSIRO Entomology, GPO Box 1700, Canberra, ACT 2601, Australia, [3]CSIRO Entomology, Locked Bag 59, Narrabri, NSW 2390, Australia, [4]Bayer BioScience, Technologiepark 38, B-9052 Gent, Belgium

Contributed Papers

Thursday, 8:00-10:00
Nihat Turan 2

VIRUSES 5

Chairs: Eric Haas Stapleton and Martin Erlandson

8:00 **195 *Mamestra configurata* nucleopolyhedrovirus strain variation at the genome sequence level.** Martin Erlandson[1], Matthew Links[1], Ajaykummar Maghodia[1], Cam Donly[2], Dwayne Hegedus[1], David Theilmann[3], [1]Agriculture and Agri-Food Canada, Saskatoon, CA; [2]Agriculture and Agri-Food Canada, London, CA; [3]Agriculture and Agri-Food Canada, Summerland, CA.

8:15 **196-STU Comparative genomics of four isolates of *Cydia pomonella* Granulovirus (CpGV)**. Karolin E. Eberle[1], Pit Radtke[2], Johannes A. Jehle[1], [1]Julius Kuhn-Institut, Bundesforschungsinstitut fur Kulturpflanzen, Institut fur biologischen Pflanzenschutz, Darmstadt, DE; [2] Laboratory of Biotechnical Crop Protection, Department of Phytopathology, Agricultural Service Center Palatinate (DLR Rheinpfalz), Neustadt/Weinstrasse, DE.

8:30 **197-STU Comparative effects of "defective" genotypes in NPV populations: Their roles in SfMNPV vs. SeMNPV**. Amaya Serrano[1], Oihane Simón[1], Delia Muñoz[2], Trevor Williams[3], Primitivo Caballero[1], [1]Instituto de Agrobiotecnología, CSIC-Gobierno de Navarra, 31192, Mutilva Baja, Navarra, ES; [2] Departamento de Producción Agraria, Universidad Pública de Navarra, 31006, Pamplona, Navarra, ES; [3]Instituto de Ecología AC, 91070, Xalapa, Veracruz, MX.

8 :45 **198 Molecular genetics of the densovirus resistance genes, nsd-1 and nsd-2, in Bombyx silkworms**. Keiko Kadono-Okuda[1], Dorington Okeyo Ogoyi[1], Kurako Kidokoro[1], Masahiro Ajimura[1], Eiichi Kosegawa[2], Yutaka Banno[3], Hideaki Maekawa[4], Yumiko Nakajima[4], Keiji Yukuhiro[1], Katsuhiko Ito[1], Kimiko Yamamoto[1], Kazuei Mita[1], [1]National Institute of Agrobiological Sciences, Tsukuba, JP; [2]National Institute of Agrobiological Sciences, Kitamori, JP; [3]Kyushu University, Fukuoka, JP; [4]University of the Ryukyus, Okinawa, JP.

9:00 **199 Dynamics of the salivary gland hypertrophy virus in laboratory colonies of *Glossina pallidipes* (Diptera: Glossinidae)**. Abd-Alla Adly[1], Kariithi Henry[1], Parker Andrew[1], Robinson Alan[1], Kiflom Musie[2], Bergoin Max[3], Vreysen Marc[1], [1]Insect Pest Control Laboratory, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, Vienna, Austria, Vienna, AT; [2]Southern Tsetse Fly Eradication Project (STEP), Ministry of Science and Technology, Akaki Kaliti Kefle Ketema W.27 K.10, Debrezeyet Road, P.O. Box 7794, Addis Ababa, Ethiopia, Addis Ababa, ET; [3]cLaboratoire de Pathologie Comparée, Université Montpellier 2, Place Eugène Bataillon, 34095 Montpellier, France, Montpellier, FR.

9:15 **200 Do persistent NPV infections in *Spodoptera exigua* affect host fitness?** Eduardo Villar[1], Cristina Virto[1], Oihana Cabodevilla[1], Rosa Murillo[1], Trevor Williams[2], Primitivo Caballero[1], [1]Instituto de Agrobiotecnología, CSIC-Gobierno de Navarra, 31192, Mutilva Baja, Navarra, ES; [2] Instituto de Ecología AC, 91070, Xalapa, Veracruz, MX.

9:30 **201 Variation in life history and flight morphological traits in speckled wood *(Pararge aegeria*) butterflies infected with a baculovirus**. Helen Hesketh[1], Melanie Gibbs[2], Casper J. Breuker[3], Hans van Dyck[2], Emma Turner[4], Rosemary S. Hails[1], [1]NERC Centre for Ecology and Hydrology, Wallingford, GB; [2] Behavioural Ecology and Conservation Group, Biodiversity Research Centre, Earth and Life Institute, Universite Catholique de Louvain, Louvain-la-Neuve, BE; [3]Evolutionary Developmental Biology Research Group, School of Life Sciences, Oxford Brookes University, Oxford, GB; [4]Department of Biology, University of York, York, GB; NERC Centre for Ecology and Hydrology, Wallingford, GB.

9:45 **202 Transmission strategy is correlated with pathogenicity and disparate virulence and productivity traits in an insect nucleopolyhedrovirus.** Oihana Cabodevilla[1], Itxaso Ibáñez[2], Oihane Simón[1], Rosa Murillo[1], Primitivo Caballero[1], Trevor Williams[3], [1] Instituto de Agrobiotecnología, CSIC-Gobierno de Navarra, 31192, Mutilva Baja, Navarra, ES; [2]Departamento de Producción Agraria, Universidad Pública de Navarra, 31006, Pamplona, Navarra, ES; [3] Instituto de Ecología AC, 91070, Xalapa, Veracruz , MX

Thursday, 8:00-10:00

VIEW POSTERS

Fungi (Authors stand by posters when not in session).

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

Thursday, 10:30-12:30
Hasan Turan

SOCIETY for INVERTEBRATE PATHOLOGYAnnual Business Meeting

Presentation by Elizabeth W. Davidson: Why do we keep coming to the SIP meeting?
Delivered by James Harper.

12:30-14:00 **LUNCH at KTU SAHIL**

13:00-14:00
Demonstration Room

Business Meeting

Student and Post Docs

Thursday, 14:00-16:00

VIEW POSTERS

Bacteria, COST 862, Virus, and Fungi
(Authors stand by posters when not in session)

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

Contributed Papers

Thursday, 16:30-18:00
Fahri Kuran

VIRUSES 6

Chairs: Karyn Johnson and Kelli Hoover

16:30 **203 Anti-viral defenses contribute to developmental resistance to LdMNPV in gypsy moth**. Kelli Hoover[1], Diana Cox-Foster[1], James Slavicek[2], James McNeil[1], [1]Penn State University, University Park, US; [2]USDA Forest Service, Delaware, US.

16:45 **204 Hemocytes proliferate in response to inactivated baculovirus**. Tiffany Chen, Eric Haas-Stapleton, California State University, Long Beach, US

17:00 **205 Baculovirus infections alter the titers of detoxification enzymes in *Spodoptera littoralis* Boisd. (Lepidoptera: Noctuidae)** Tugba Erdogan[1], M. Oktay Gurkan[1], Umut Toprak[2], [1]University of Ankara, Ankara, TR; [2]University of Saskatchewan, Saskatoon, CA.

17:15 **206 Effects of silencing apoptosis regulatory genes on Sindbis virus replication and dissemination in *Aedes aegypti***. Hua Wang, Taryn Penabaz, Rollie Clem, Kansas State University, Manhattan, US.

17:30 **207 Prospects for managing turfgrass insect pests with baculoviruses** Andrea J. Bixby , Daniel A. Potter, Dept. of Entomology University of Kentucky
Lexington, KY.

Contributed Papers

Thursday, 16:30-18:15
Nihat Turan 1

BACTERIA 5

Chair: Hyun-Woo Park and Gregory T. Sullivan

16:30 **208 Genetic assessment of the virulence factors of *Serratia proteamaculans* strains that cause atypical disease symptoms in the grass grub *Costelytra zealandica***. Mark Hurst, Joanne Calder, Joanne Calder, AgResearch, Lincoln, NZ.

16:45 **209 Bacteria isolated from overwintering *Hyphantria cunea* (Lepidoptera: Arctiidae) pupae as potential entomopathogens**. Gregory T. Sullivan[1], H. Murat Aksoy[2], Sebahat K. Ozman-Sullivan[3], Ismail Karaca[4]. [1]Suleyman Demirel University, Faculty of Agriculture, Department of Plant Protection, Isparta; Ondokuz Mayis University, OYDEM, Samsun, TR; [2]Ondokuz Mayis University, Faculty of Agriculture, Department of Plant Protection, Samsun, TR; [3]Ondokuz Mayis University, Faculty of Agriculture, Department of Plant Protection, Samsun, TR; [4]Suleyman Demirel University, Faculty of Agriculture, Department of Plant Protection, Isparta, TR.

17:00 **210 Diversity and transmission of *Wolbachia* in spider mites**. Vera I.D. Ros[1], Vicki M. Fleming[2], Johannes A.J. Breeuwer[2], [1]University of Amsterdam, Institute for Biodiversity and Ecosystem Dynamics, Amsterdam; Wageningen University, Laboratory of Virology, Wageningen, NL; - [2]University of Bath, Department of Biology and Biochemistry, GB.

17:15 **211 Identification of a biosynthesis gene cluster involved in *Pseudomonas entomophila* virulence toward *Drosophila***. Onya Opota[1], Isabelle Vallet-Gely[2], Alexey Novikov[3], Martine Caroff[4], Bruno Lemaitre[1], [1]Global Health Institute, Ecole Polytechnique Fédérale Lausanne (EPFL), Lausanne, CH; [2]Centre de Génétique Moléculaire, CNRS, Gif sur Yvette, FR; [3] Institut de Génétique et Microbiologie UMR CNRS 8621, Université Paris Sud 11, Paris, FR; [4]Structure et Activités des Endotoxines, Institut de Génétique et Microbiologie, Université de Paris Sud-XI , Paris, FR.

17:30 **212 The effects of entomopathogenic bacteria on *Thaumetopoea pityocampa* (Lepidoptera: Thaumetopoeidae).** Sebahat K. Ozman-Sullivan[1], Hazan Alkan-Akıncı[2], H. Murat Aksoy[1], Heval Ocal[1], Nuray Celik[1], [1]Ondokuz Mayis University,Faculty of Agriculture, Department of Plant Protection, Samsun, TR; [2]Artvin Coruh University, Faculty of Forestry, Department of Forestry Engineering, Artvin, TR.

17:45 **213 Evidence of the involvement of 358E, 498A and 571C in the toxicity and binding of a new Cry1Ac-type protein of *Bacillus thuringiensis* to *E. kuehniella* receptors**. Imen Saadaoui[1,] Nabil Miled[2], Lobna Abdelkefi Misrati[1], Slim Tounsi[1], Souad Rouis,[1,3], Samir Jaoua[1,3], [1]Laboratory of Biopesticides, Centre of Biotechnology of Sfax, Tunisia. [2]Laboratoire de Biochimie et de Génie Enzymatique des Lipases, ENIS, Sfax, Tunisia. [3]Biological and Environmental Sciences Department, College of Arts and Sciences, Qatar University, Doha, Qatar.

18:15 Buses leave for hotels

18:30 Buses return from hotels to KTU SAHIL

19:00-20:00 Cocktail hour

20:00-00:30 BANQUET and AWARDS CEREMONY

22:30-00:30 Buses return to hotels

We hope to see you in Halifax, Canada for SIP 2010!

ABSTRACTS 2010

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
136 indicates abstract number for ORAL presentation
N-05 indicates abstract number for POSTER presentation

Monday July 12, 2010

Plenary Symposium

Monday, 10:30-12:30

Hasan Turan

Biology of the Tsetse fly: Interactions with Parasites, Pathogens and Symbionts

Convener: Dr. Adly Abd-Alla. FAO/IAEA Agriculture and Biotechnology. Vienna, Austria

Plenary Session Monday, 10:30 1

Tsetse distribution and biology, and options and strategies for vector control

Udo Feldmann
Insect Pest Control Section, Joint FAO/IAEA Division, Vienna, AT
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Tsetse flies are unique in their biology, physiology and regarding their function as cyclical vectors of trypanosomes. Tsetse flies affect human and animal health and sustainable rural development in Africa and are often referred to as the 'poverty insect'. Both sexes are obligatory blood feeders. Tsetse females are larviparous and deposit a mature third instar lara every nine days, which explains – in comparison to many other pest insects – the relatively low population densities of tsetse in the field. Flight energy is based on a proline - analine conversion cycle, which permits flight durations of about five minutes at a time. Tsetse and the disease they transmit, human African trypanosomosis (or sleeping sickness) and animal African trypanosomosis (or nagana) exist in 36 countries in tropical and sub-tropical sub-Saharan Africa, covering an area of about 8.7 million km². 60 million people and some 50 million cattle, and additional other livestock, living in tsetse infested areas are at risk of the disease. There are more than 30 tsetse species and subspecies, belonging to three major groups, i.e. riverine, forest and savannah species. Each species has slightly different climatic requirements and preferences regarding vertebrate hosts, ranging from reptiles over various wildlife and livestock to humans. The management or, if possible, sustainable elimination of the tsetse and trypanosomosis problem requires a thorough exercise of standardised baseline data collection and feasibility assessment, based on which a combination of suitable tsetse control tactics can be applied as part of an area-wide integrated pest and disease management campaign.

Keywords: Trypanosome, Poverty insect, African trypanosomosis

Plenary Session Monday, 11:00 2

Tsetse-transmitted trypanosomes – their biology and disease impact

Peter Holmes
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Tsetse-transmitted trypanosomes (genus *Trypanosoma*) are a major constraint on human and animal health in huge areas of sub-Saharan Africa. In domestic animals the major pathogenic species are *T congolense*, *T vivax*, and *T brucei brucei*. The trypanosomes that cause tsetse-transmitted trypanosomiasis (sleeping sickness) in people are *T brucei rhodesiense* and *T brucei gambiense.* Most tsetse transmission is cyclic and begins when blood from a trypanosome infected human or animal is ingested by the tsetse fly. *T brucei* spp migrate from the gut to the proventriculus to the pharynx and eventually to the salivary glands; the cycle for *T congolense* stops at the hypopharynx, and the salivary glands are not invaded; the entire cycle for *T vivax* occurs in the proboscis. In humans the primary clinical signs are associated with invasion of the brain and associated neurological changes whilst in animals anaemia and wasting are the major feature. Following the description of the life-cycle over 100 years ago many different methods of treatment and control have been developed but unfortunately the disease continues to affect almost the same area of Africa (approx 9 million sq kms). Most success to date has been achieved in situations in which various methods of control have been used in an area-wide and integrated way. However more effective and safer drugs and better methods of tsetse control are urgently needed.

Keywords: Trypanosoma, Sleeping sickness

Plenary Session Monday, 11:30 3

Influences of the symbiotic fauna on host physiology

Serap Aksoy
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Tsetse flies, sole vectors of African trypanosomes, have coevolved with multiple symbionts: mutualistic endosymbiont *Wigglesworthia*. commensal *Sodalis* and parasitic *Wolbachia*. The symbiotic bacteria influence important host physiological processes that range from nutrient provisioning to immune competence and reproductive outcomes. It has been possible to maintain tsetse lines through dietary supplementation that either lack *Wigglesworthia* or all three endosymbionts. Absence of *Wigglesworthia* alone compromises tsetse's immune resistance to pathogenic trypanosomes. Absence of *Wolbachia* bacterium indicates the expression of strong Cytoplasmic Incompatibility phenomenon in tsetse. Results indicate that females NOT infected with *Wolbachia* are unable to produce successful progeny when mated with and *Wolbachia* infected male. This presentation will discuss the highly integrated nature of symbiosis in tsetse and its potential impact of vector control methods.

Keywords: Tsetse flies, Wigglesworthia

Plenary Session Monday, 12:00 4

Salivary Gland Hypertrophy Virus (SGHV): Impact on tsetse rearing and potential virus management strategies

Abd-Alla Adly

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Many species of tsetse flies (Diptera: Glossinidae) are infected with a virus that causes salivary gland hypertrophy (SGH) and flies with SGH symptoms have a reduced fecundity and fertility. The prevalence of SGH in wild tsetse populations is usually very low (0.2-5%) but higher prevalence rates have been observed (15.2%). The successful elimination of a *Glossina austeni* population from Unguja Island (Zanzibar) using an area-wide integrated pest management approach with a sterile insect technique (SIT) component (1994-1997), encouraged several African countries to include SIT in their national tsetse control programs. A large facility to produce tsetse flies for SIT application in Ethiopia was inaugurated in 2007. To support this project, a *Glossina pallidipes* colony originating from Ethiopia was successfully established in 1996 at the Insect Pest Control Laboratory (former Entomology Unit) of the FAO/IAEA Agriculture and Biotechnology Laboratories in Seibersdorf, Austria, but up to 85% of adult flies displayed symptoms of SGH. As a result, the colony declined and became extinct by 2002. The difficulties experienced with the rearing of *G. pallidipes*, epitomized by the collapse of the *G. pallidipes* colony originating from Ethiopia, indicates the urgent need for a management strategy of the Salivary Gland Hypertrophy Virus (SGHV) for this species. To identify suitable management strategies, the virus isolated from *G. pallidipes* (GpSGHV) was recently sequenced and research was initiated on virus transmission and pathology. Different approaches to prevent virus replication and its horizontal transmission during blood feeding have been initiated and their preliminary results will be presented. These include the use of antiviral drugs such as Acyclovir and Valacyclovir added to the blood for feeding or the use of antibodies against SGHV virion proteins. In addition, preliminary attempts to silence the expression of this protein using RNA interference are described.

Keywords: Tsetse, Salivary Gland Hypertrophy Virus (SGHV), Transmission, Virus Management

Symposium Fungi

Monday, 14:00-16:00
Hasan Turan

Environmental Change and Entomopathogenic Fungi

Organizers: Helen Hesketh and Helen Roy

Symposium Monday, 14:00 5

Influence of environmental temperature on insect-pathogen and insect-parasite interactions

Matthew Thomas
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There is considerable interest in understanding the effects of environmental factors on host-pathogen and host-parasite interactions, both to better understand present-day dynamics and to predict the consequences of future climate change. Most studies examining the effects of the environment consider relatively coarse measures such as mean monthly temperature recorded from met stations. However, the actual conditions experienced by insects and their parasites can differ markedly from these ambient conditions. Using varied examples of flies, mosquitoes, fungi, malaria and arboviruses, we illustrate how subtle variations in microclimate, together with additional influences of thermal behavior, can alter the outcome of host-parasite/pathogen interactions relative to ambient conditions. These examples highlight a need for greater ecological understanding of how infectious organisms and their insect hosts respond to the natural environment.

Keywords: Mosquitoes, malaria, virulence, resistance, fever, climate change

Symposium Monday, 14:30 6

Modelling environmental gradients in host-pathogen systems

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Patterns in species abundance have had a long interest for ecologists. However, the environmental drivers, such as biotic and abiotic factors, of these patterns are often debated and difficult to generalise. Moreover, the way in which environment affect species is often varied, which is particularly true for the tightly coupled host-pathogen dynamics. In this paper we use a spatially explicit host-pathogen metapopulation model, where environmental gradients affect host and pathogen life-histories independently, to ascertain how the environment shapes abundance.

We show that when the host and pathogen environmental gradients overlap then we observe greater host suppression at the optimal spatial location than that at sub-optimal locations, thus giving rise to abundance patterns that contrast current observations and predictions. This result contrasts scenarios where the optimal environment for the host does not coincide with that of the pathogen. In addition, we show that the rate of migration across the environmental gradient has surprising effects on both host and pathogen abundance. In particular, our model predicts that, in contrast to current theory, increased host dispersal leads to decreased pathogen abundance, whilst greater pathogen dispersal leads to an increase or decrease in pathogen abundance, which is mediated by host resource competition.

The predictions of our model are discussed with applications to conservation, pest management and climate change.

Keywords: Fungal entomopathogens, metapopulation, mathematical model, insect and pathogen abundance, dispersal

Symposium Monday, 15:00 7

Entomopathogenic fungi and invasional meltdown

Helen Roy
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Invasive non-native (alien) species are considered to be one of the greatest threats to biodiversity. The rate of new species arriving in many countries is increasing. Approximately one percent of non-native species are predicted to be problematic; the effects of these few can be extensive. Understanding the interactions between invasive non-native species and other species, within an invaded range, is challenging but essential, particularly for quantifying biodiversity impacts. In this presentation I will consider the interactions between entomopathogenic fungi and invasive non-native species using the harlequin ladybird, *Harmonia axyridis*, as a model. *Harmonia axyridis* is considered to be an invasive non-native species in Europe, America and Africa. This charismatic ladybird is a top-predator within the aphidophagous guild; a community which comprises both native and non-native species. The concept of "invasional meltdown" is used to describe synergistic interactions among invasive non-native species which lead to accelerated impacts on native ecosystems. Could *H. axyridis* provide insights on invasional meltdown?

Keywords: Invasive non-native species, fungal entomopathogen

Symposium Monday, 15:30 8

Life cycles of specialist insect pathogenic fungi: Can we expect any effects from environmental changes?

Jørgen Eilenberg
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Insect pathogenic fungi exhibit different types of life cycles. These may include production of either sexual or asexual spores in or on infected hosts and these spore types may be adapted to different conditions. For example, thick walled resting spores are important for winter survival, while conidia are important for epizootic development during the cropping season. Each part of the fungus life-cycle can be influenced by changes in environmental conditions, for example a rise in temperature due to global warming. Concerning specialist insect pathogenic fungi the dependency on the presence of one host species or few host species from closely related taxa provides a special challenge for the fungi: even small changes in the conditions for the host or for the pathogen may have a significant effect. These elements will be discussed with emphasis on examples on specialist insect pathogenic fungi from Entomophthorales and their interactions with their insect host species

Keywords: Fungi, Entomophthorales, life cycles, environmental effects

Contributed papers

Monday, 14:00-16:00
Fahri Kuran

VIRUSES 1

Chairs: Hu Zhihong and Agah Ince

Contributed Paper Monday, 14:00 9

Genome organization and expression of the translation products of *Providence virus*: The type member of a new family of small insect RNA viruses?

Cheryl Walter[1]; Fiona Pringle[2]; Ritah Nakayinga[3]; Pablo de Felipe[4]; Martin Ryan[4]; Andrew Ball[2]; Rosemary Dorrington[3]
[1]Institute of Molecular and Cellular Biology, University of Leeds, Leeds, GB; [2]Dept. of Microbiology, University of Alabama at Birmingham, Birnimgham, Alabama, US; [3]Department of Biochemistry, Microbiology and Biotechnology, Rhodes University, Grahamstown, ZA; [4]Centre for Biomolecular Sciences, University of St. Andrews, St Andrews, Scotland, UK
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The T*etraviridae* are a family of small, non-enveloped positive sense (+ve) RNA viruses that exclusively infect larvae of lepidopteron insects, with a narrow host range infecting larval midgut cells. The *Tetraviridae* are classified into two genera according to their genome organisation, namely the monopartite betatetraviruses or bipartite omegatetraviruses. *Providence virus (*PrV), the only tetravirus able to replicate in insect tissue culture and was classified as a betatetravirus due to the characteristic *T*=4 symmetry of its capsids, maturation dependent autoproteolytic cleavage of the capsid protein precursor (VCAP) and its monopartite genome organisation. We present the genome organization of PrV and data on the translation and co-translational processing of viral proteins in *vitro* and in *vivo*. As with other betatetraviruses, PrV encodes a viral replicase (p104) followed by VCAP (p81) also a unique, third ORF (p130) of unknown function. Both p130 and p81 encode functional 2A-like processing sites at their N-termini while p104 encodes a read-through stop signal resulting in the translation of two overlapping proteins (p40 and p104) from the same ORF. Phylogenetic analysis shows that the PrV VCAP is more closely related to those of the omegatetraviruses than the betatetraviruses and that the replicase is most closely related to members of the *Umbraviridae* and *Tombusviridae*, both families of (+ve) RNA plant viruses. Taken together, our data lead us to propose that the *Tetraviridae* be reclassified and that PrV becomes the type member of a new virus family, the *Prototetraviridae*

Keywords: Providence virus, tetravirus, genome organization

Contributed Paper Monday, 14:15 10

Subcellular targeting of the *Helicoverpa armigera stunt virus* replicase: evidence that tetraviruses replicate in association with membranes derived from the endocytic pathway

James Short[1]; Rosemary Dorrington[1]
[1]Department of Biochemistry, Microbiology and Biotechnology, Rhodes University, Grahamstown, ZA
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The T*etraviridae* are a family of small, non-enveloped positive sense RNA viruses that exclusively infect the larvae of lepidopteron insects. They have an unusually narrow host range and a high degree of tissue tropism limited to larval midgut cells. *Helicoverpa armigera stunt virus* (HaSV), which infects agriculturally important heliothine species, has attracted much interest as a potential biocontrol agent, but the absence of an efficient *ex vivo* virus production system has hampered the development of commercial applications of HaSV. Our interest lies in developing a fundamental understanding of tetraviral replication with the view to overcoming the barriers to viral replication in tissue culture. Our approach has been to identify the site of viral replication in host cells by co-localising replication proteins with subcellular compartments using fluorescence microscopy. We describe the characterisation of targeting domains that result in subcellular localisation of the HaSV replicase (REP) in mammalian and insect tissue culture cells. Expression of REP with enhanced green fluorescent protein fused at its C-terminus resulted in a punctuate distribution of the fusion protein, associated with modified membranes derived from the endocytic pathway. Analysis of the distribution of deletion mutants and selected site-directed mutants has identified at least two novel protein targeting domains, one at the N-terminus and the other within the C-terminal region of the RNA-dependant RNA polymerase domain. Our data provides new insight on the molecular mechanisms of infection and the site of replication in infected host cells.

Keywords: Tetravirus, replication, endocytic membranes

Contributed Paper Monday, 14:30 11

Induction of an IAP antagonist in *Culex quinquefasciatus* larvae in response to infection by the baculovirus CuniNPV

James Becnel[1] ; Liu Bo[2] ; Zhang Yanping[2] ; Zhou Lei[2]
[1]Center for Medical, Agricultural and Veterinary Entomology, USDA/ARS, Gainesville, US; [2]Dept. of Molecular Genetics and Microbiology, Shands Cancer Center, University of Florida, Gainesville, US;

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CuniNPV is a member of the Dipteran–specific baculoviruses in the genus Deltabaculovirus that specifically infects mosquito larvae within the genus *Culex* while species of *Aedes* and *Anopheles* are refractory. Infections are restricted to the nuclei of larval midgut epithelial cells with transmission dependent upon the presence of certain divalent cations such as magnesium. Recently, reaper-like IAP-antagonist have been identified in *Anopheles* and *Aedes* mosquitoes and designated Michelob_x (*mx*). The functional domain (the IAP-binding motif) was very well conserved and the functional mechanism of *mx* appears to be very similar to that of reaper. In order to determine if reaper-like genes are involved in the pro-apoptotic response to viral infection, expression of mx was measured in larval mosquitoes following exposure to CuniNPV. There was not a significant increase of *mx* expression before 8hr p.i. but the level of *mx* expression continued to increase throughout the infection period and at 48 hr p.i.was about 10 times higher than the uninfected controls. The induction of mx did not result in apoptosis but rather necrosis indicating that CuniNPV prevents apoptosis despite the very high level of *mx* expression. It is possible that *CuniNPV* utilizes a yet unknown, but powerful, mechanism to block the apoptotic pathway downstream of *mx* activation.

Keywords: Baculovirus, mosquito, Deltabaculovirus, Culicidae IAP antagonist, apoptosis

Contributed Paper Monday, 14:45 12-STU

The proteome of *Glossina pallidipes* Salivary Gland Hypertrophy Virus (*Hytrosaviridae*) virions

Ikbal Agah Ince[1]; Henry M. Kariithi[1]; Sjef Boeren[2]; Jacques Vervoort[2]; Max Bergoin[3]; Just M. Vlak[1]; Adly M. M. Abd-Alla[4]; Monique M. van Oers[1]
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Tsetse flies (*Glossina pallidipes*) can be infected by a virus causing salivary gland hypertrophy (SGH). The genome of the causative virus (GpSGHV) has recently been sequenced containing 161 open reading frames (ORF). A potential strategy to control or eradicate viral infections in tsetse fly colonies for 'sterile insect technique' (SIT) programs is the neutralization of GpSGHV infections by adding specific antibodies against virion proteins to the blood meal. Two immunodominant virion proteins, about 130 kDa and 50 kDa in size, were identified by western analysis using antiserum against whole GpSGHV virions. To identify the encoding genes, the proteome of GpSGHV virions was determined. GpSGHV proteins were separated by gradient SDS-PAGE and the protein bands excised, digested and characterized by liquid chromatography tandem mass spectrometry (LC-MS/MS). Sixty-one proteins were identified in the GpSGHV proteome including all peroral infectivity factors (PIFs + P74). The twenty-nine proteins are found in the proteome of *Musca domestica* (Md) SGHV, another hytrosavirus, of which twelve are present in the GpSGHV proteome. On the basis of size, specific GpSGHV virion proteins were expressed for production of mono-specific polyclonal antibodies. The products of GpSGHV ORF10 (unique) and ORF96 appeared to be the most immunodominant

antigens and therefore good candidates as starting point for immune intervention studies of viral infections in tsetse fly colonies. The data obtained in this study also provide further impetus for future studies on the function of GpSGHV virion proteins, more specifically the role of PIF proteins in the *per os* SGHV infection of tsetse flies.

Keywords: Tsetse, Salivary gland hypertrophy, *Glossina pallidipes*, Sterile insect technique, Antibodies, Viral Proteins, Mass spectrometry, Proteome

Contributed Paper Monday, 15:00 13

Delineating the baculovirus secretome

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Secreted proteins that are encoded in viral genomes can influence the interactions between a pathogen and its host at a level far beyond that of the infected cell. Mammalian cells infected with poxviruses, for example, secrete viral immunomodulatory proteins that debilitate the host's capacity to counter infection. Among the insect baculoviruses, the best known secreted viral protein is EGT, which can disrupt host development and increase progeny virus yield. EGT is one of only three baculovirus proteins that are known to be secreted, and these three are often described (in, arguably, somewhat disparaging terms) as being the products of 'auxiliary genes'. Here I take a bioinformatic approach, using selected viral genomes, in an attempt to identify complete sets of baculovirus genes that are likely to encode secretable proteins. The strategy is simple. Proteome-level amino acid sequences are first scrutinized using SignalP 3.0 for predicted signal peptide sequences at their N termini; around 20% (maximum, 25%; minimum, 12%) of the proteins encoded by each genome have putative signal peptides. These preliminary lists are then filtered to remove proteins whose known localization is intracellular; unsurprisingly, many of these are viral envelope components. Putative transmembrane domains are finally identified using Phobius. For each viral genotype, the residual collection of proteins represents a candidate secretome. I will describe the derivation of these proposed sets of secreted proteins, and discuss what they may tell us about virus-host interactions and host specificity.

Keywords: Baculovirus, secretion, signal peptide

Contributed Paper Monday, 15:15 14-STU

Identification of an unusual structure found in Sf9 cells transfected with AcMNPV mutants defective in nucleocapsid assembly

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The family Baculoviridae is a highly selective pathogen in arthropods, mainly in insects of the order Lepidoptera. *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) *ac54* gene is a highly conserved gene in baculovirus genome. In this study, ET recombination and Bac-to-Bac system was used for the first time to generate an *ac54*-knockout virus. Microscopy and titration assays showed that *ac54*-knockout virus can not produce infectious budded virus, whereas repair of *ac54* can rescue the defect back to wild-type levels. Electron microscopy showed that large amount of vesicles and electron-lucent tubular structures containing capsid protein VP39 were found in ring zone area, indicating that the loss of *ac54* directly leads to interruption of nucleocapsid assembly. Novel round, electron-dense bodies were formed associated with virogenic stroma structure, which has been reported in a previous study on the temperature-sensitive mutant of AcMNPV tsN1054-infected cells. Interestingly, besides *ac54*-knockout virus, other mutants such as *pp78/83*-knockout virus, *p6.9*-knockout virus, *vp39*-knockout virus, *ac53*-knockout virus and *38k*-knockout virus, which all fail to form nucleocapsid in the nuclei of corresponding mutant-infected cells, can also induce the formation of the unusual structure, suggesting that the emergence of the structure is due to incomplete nucleocapsid package and assembly. To indentify the components of the structure, immuno-electron microscopy were performed by using antibodies against several baculovirus structural proteins.

Keywords: AcMNPV, ac54, virogenic stroma; nucleocapsid

Contributed Paper Monday, 15:30 15

Histopathology of European decapod crustaceans exposed to White Spot Syndrome Virus (WSSV)

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White Spot Disease (WSD) is caused by a virus (WSSV) and can lead to devastating losses in tropical shrimp farming regions. The virus has been responsible for mortalities of up to 100 % in commercial shrimp farms and has also been detected in wild crustaceans in tropical and sub-tropical regions. Although WSSV is most commonly associated with penaeid shrimp farmed in warm waters, the virus is also able to infect and kill a wide range of other decapod crustaceans from temperate regions, including lobsters, crabs, crayfish and shrimp. Although not discovered naturally in populations of wild European crustaceans, in our studies, it has been transmitted to a range of ecologically and commercially important marine crustaceans maintained at temperatures conducive with European aquatic habitats. Here we present an overview of the differing pathologies presented by the European lobster, *Homarus gammarus*, Edible crab, *Cancer pagurus* the Norwegian Lobster, *Nephrops norvegicus* and the shore crab, *Carcinus maenas* following exposure to WSSV via feeding and injection. This presentation will also examine the pre-existing pathogen profile of these hosts and discuss how this may have an influence on individual and population level susceptibility to WSSV infection, and to the resultant disease that it may cause.

Keywords: White Spot Syndrome Virus, Crustacean

Contributed Paper **Monday, 15:45 16**

Gill-associated virus and protein subunit vaccination in *Penaeus monodon*

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Invertebrates lack the classical adaptive immune responses found in vertebrate animals. However, recent studies have suggested that some invertebrates, including penaeid prawns, have immune defences that may include specificity and memory, which are hallmarks of the adaptive immune response. This phenomenon has been well documented for both prawns and other crustaceans that are susceptible to *White spot syndrome virus* (WSSV). Prior exposure or "vaccination" with recombinantly expressed viral envelope proteins can protect prawns from WSSV induced mortality. To date this phenomenon has only been described for WSSV. Here we investigated the generality of this phenomenon by vaccinating *Penaeus mondon* with recombinantly expressed protein domains from both the envelope and nucleocapsid proteins of *Gill-associated virus* (GAV). The prawns were challenged with virus 2 days after vaccination and booster with either proteins or non-specific protein controls. The prawns were monitored for mortality and samples taken to assay virus loads. There was no significant difference in either mortality or virus accumulation between prawns vaccinated with either GAV protein subunits or mock vaccinated. These results suggest that the efficacy of protection following vaccination may vary between viruses.

Keywords: White spot syndrome virus, Gill-associated virus, Vaccination

Contributed Papers
Monday, 14:00-16:00
Nihat Turan 1

BACTERIA 1

Chairs: Juan Luis Jurat-Fuentes and Hyun-Woo Park

Contributed Paper **Monday, 14:00 17**

Comparison of virulence and virulence gene expression in *B. cereus* groupe bacteria during infection of *Galleria mellonella*

Kader Rebaine[1]; **Agnes Rejasse[1]**; **Christophe Buisson[1]**; **Eugenie Huillet[1]**; **Claudia Bevilacqua[1]**; **Christina Nielsen-LeRoux[1]**
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Bacillus cereus sensu stricto (Bc) and *Bacillus thuringiensis* (Bt) are parts of the *Bacillus cereus* sensu lato group. Bt is the entomopathogen used as due to the presence of Cry toxins Cry.The gnes encoding these toxins are on plasmids and they permit to distinguish Bt from Bc. Bc is considered an opportunistic human pathogen. These bacteria can produce common various chromosomal virulence factors many of which are part of the PlcR regulon. (Agaisse et al. 1999, Gohar et al.2008). A plcR mutant has strongly reduced virulence in the infection model Galleria mellonella (Salamitou et al. 2000). Few studies have so far investigated on the expression of the important PlcR regulated factors (enterotoxins, metalloproteases etc.) during infection. In this study we have compared the virulence of various Bt (from commercial products) and Bc strains (clinical origin) and one animal probiotic strain in G. mellonella 5th instars. Insect were infected orally with spores and vegetative bacteria and mortality was recorded after incubation at 37°C at 24, 48 hours post infection. Some strain differences were shown, but Bc strains were not more virulent than Bt strains. To get knowledge into the expression of "supposed" virulence genes in the insect midgut, RT-PCR and Q-RT (when possible) analysis on bacteria extracted from the midgut of the infected larvae were performed. Preliminary results show that genes from the PlcR regulon are expressed differently in the midgut compared to i medium and that not all tested (Hbl, Nhe, CytK) PlcR regulated genes follows the same behaviour. This is in accordance with former studies conducted with gfp-promoter fusion studies in a Bt strain 407.

Keywords: *Bacillus thuringiensis*, *Bacillus cereus*, virulence, Q-RT-PCR, *in vivo* expression, *Galleria mellonella*

Contributed Paper **Monday, 14:15 18-STU**

A novel approach for producing random mutation library with prospective base substitution rate for cry genes directed evolution

Ming Liu[1]; **Changlong Shu[1]**; **Chengxi Lin[1]**; **Fuping Song[1]**; **Dafang Huang[1]**; **Jie Zhang[1]**
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The Cry toxins, encored by cry genes of *Bacillus thuringiensis,* are specific for insects, and are thus safe for humans and domestic animals, many cry genes have been cloned and applied in the development of transgenic plants. But, the low toxicity of some Cry toxins limited its application. At present, directed evolution had been considered as one promoting way to solve the issue. However, it is reported that toxins with enhanced bioactive properties were rare or nonexistent from the high mutation rate libraries. Therefore, we investigated the possibility of the direct evolution of cry toxins by constructing the mutation libraries with prospective base substitution rate. In this report, the *cry1Ie* gene was used as a model. *Taq* DNA polymerase has a high intrinsic error rate between 2×10^{-4} and 8×10^{-6} error/nucleotide synthesized and been used widely in generate mutation library. In the experiment, to generating the prospective base substitution rate libraries, we using real time PCR as a monitor to control the nucleotide synthesis. We constructed three libraries with the prospect that the libraries of base-substitutions in DNA molecules were 1 (Library I), 2 (Library II) and 3 (Library III) respectively. 100 random clones from each library were selected for subsequence analysis. The result shown that the number of mutated clone of Library I, Library II and Library III was 59, 93 and 98 respectively; the number of nonsense mutation of Library I is 36, Library II is 43, Library III is 50, the number of missense mutation of Library I is 13, Library II is 41, Library III is 38. In general, the 2

base-substitutions in DNA molecules are best, and the high mutation rate is not fit for the directed evolution. Furthermore, we evaluated the toxicity of the 92 mutants of Cry1Ie against *Plutella xylostella*, and 12 of them had an improved toxicity and the ratio of mutants with improved toxicity is considerable. In conclusion, the low mutation rate is more suitable for directed evolution of Cry toxin.

Keywords: Prospective base substitution rate; cry; directed evolution

COST862 Monday, 14:30 19

Modification of nucleotide sequences between RBS and start codon in *Bacillus thuringiensis* resulted in increased level of cry2Ac mRNA

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Insecticidal Cry toxins that *B. thuringiensis* produce during the sporulation have been employed for biological control of pest insects through formulations. Various strategies have been used to increase the yield of these toxins per unit medium. Cry2Ac has dual toxicity against certain lepidopterous and dipterous insects. The *cry2Ac* gene appears as a third gene in an operon that consists of three open reading frames (*orf*s). However, crystallization of the Cry2Ac protein requires the second ORF (ORF2) in the *cry2Ac* operon as a scaffolding protein. In order to enhance activity of the Cry2Ac toxin from HD29 strain of *B. thuringiensis* subsp. *galleriae* (*Bacillus* Genetic Stock Center ID 4G5; Serotype 5a5b), the *cry2Ac* gene was overexpressed in *B. thuringiensis* by genetic modification. The number and the sequence of the nucleotides between ribosome-binding sequence (RBS) and start codon (ATG) were modified and the *cry2Ac* genes with modified upstream sequences were cloned in pSTAB shuttle expression vector containing the *cyt1A* promoters from *B. thuringiensis* subsp. *israelensis* combined with the STAB-SD sequence from *B. thuringiensis* subsp. *morrisoni* strain tenebrionis. The resulting plasmids were introduced in 4Q7, an acrystalliferous mutant strain of *B. thuringiensis* subsp. *israelensis* and the Cry2Ac production of recombinant strains was confirmed by SDS-PAGE. The mutants were analyzed for mRNA level through realtime PCR. The level of mRNA varied with changes in number and sequence of nucleotides between RBS and ATG. Consequently, synthesis of Cry2Ac varied greatly throughout the recombinants with up to 10-fold increase compared with the wild-type Cry2Ac. Accordingly, crystals of the over-produced Cry2Ac were much larger than those of the wild-type.

Keywords: Cry2Ac, RBS, mutations, mRNA level

Contributed Paper Monday, 14:45 20

cDNAs and mRNA levels of Aminopeptidase N protein genes from *Ostrinia furnacalis* strains with different susceptibilities to *Bt* toxins

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Four cDNA fragments (*Ofapn*1, *Ofanp*2, *Ofapn*3, and *Ofapn*4) encoding full-length aminopeptidase N (APN) proteins were cloned from ACB-BtS and ACB-AbR, a Cry1Ab toxin protein susceptible and a resistant strains of *Ostrinia furnacalis* (Guenée). The nucleotide sequences have been deposited in the GenBank database with accession numbers GQ927480 (*Ofapn*1s), GQ927479 (*Ofapn*1r), EU564811 (*Ofapn*2s), EU826127 (*Ofapn*2r), EU137839 (*Ofapn*3s), EF538427 (*Ofapn*3r), EU571948 (*Ofapn*4s) and EU826126 (*Ofapn*4r). *Ofapn*1, *Ofapn*2, *Ofapn*3, and *Ofapn*4 cDNA fragments amplified from both *O. furnacalis* stains contained 2627, 3541, 3591, and 3001 nucleotides, including 2367-, 2823-, 3045-, and 2856-nucleotide open reading frames that encoded 788, 940, 1014, and 951 amino acid residues, respectively. These deduced amino acid sequences shared some common structural features of lepidopteran APN proteins, including the consensus zinc-binding motif $HEXXHX_{18}E$ and a highly conserved GAMEN motif, a cleavable N-terminal signal peptide with 18 amino acids, and a glycosylphosphatidylinositol (GPI) anchor signal peptide with 22 amino acids in C-terminal. Each of the four putative protein sequences was most similar to those from *O. nubilalis* with 96.6% sequence identity, respectively. Compared with ACB-*Bt*S, there were 26, 49, 40, and 31 nucleotide differences in the open reading frame of *Ofapn*1, *Ofapn*2, *Ofapn*3, and *Ofapn*4 in ACB-AbR, which resulted in 10, 5, 10, and 12 amino acid differences in the deduced protein sequences. Quantitative RT-PCT analyses showed that mRNA expressing levels of *Ofapn*1, *Ofapn*2, *Ofapn*3, and *Ofapn*4 in fifth instar larvae midguts were 2.03-, 1.61-, 2.69-, and 2.62-fold higher in ACB-AbR than in ACB-*Bt*S.

Keywords: *Ostrinia furnacalis*, Cry1Ab resistance, Aminopeptidase N, cDNA, mRNA

Contributed Paper Monday, 15:00 21

Proteomic characterization of the Cry1Ac-induced secretome in mature midgut cell cultures from *Heliothis virescens* larvae

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Cry1Ac is the most active *Bacillus thuringiensis* toxin against larvae of *Heliothis virescens*. Intoxication of *H. virescens* primary midgut cell cultures with Cry1Ac results in secretion by midgut cells of growth factors that activate midgut regeneration. This regenerative mechanism has been hypothesized to be involved in resistance to diverse Cry toxins in strains of *H. virescens*. The goal of our project is to identify the

specific growth factors involved in this response and alterations in this response that correlate with resistance to diverse Cry toxins. We used *H. virescens* primary mature midgut cell cultures to obtain the Cry1Ac-induced secretome: all the proteins secreted by mature cells during intoxication. Using a proteomic approach we identified proteins in the Cry1Ac-induced secretome and compared it to secretomes from control treatments. Furthermore, through relative quantification, we were able to determine specific proteins with altered expression in the secretome after Cry1Ac intoxication. Our results provide the first insight into specific proteins involved in communication between mature and stem cells during Cry intoxication to regulate the regenerative response in the larval midgut.

Keywords: Proteome, Cry1Ac, *Heliothis virescens*, midgut cells

Contributed Paper Monday, 15:15 22

Mutational analyses of loops in domain II of *Bacillus thuringiensis* mosquitocidal Cry4Aa toxin

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Cry4Aa produced by *Bacillus thuringiensis* is a dipteran-specific toxin and is of great interest for developing a bioinsecticide to control mosquitoes. Recent years, to characterize a potential receptor-binding site, namely loops 1, 2 and 3 in domain II of Cry4Aa, we have constructed a series of Cry4Aa mutants and analyzed their mosquitocidal activity. The analysis using Cry4Aa mutants in which one of the loops was replaced with either of the other two loops revealed that the replacement of loop 2 caused a significant loss of mosquitocidal activity, but not loops 1 and 3. This suggested that loop 2 was essential for the mosquitocidal activity of Cry4Aa. On the other hand, another series of Cry4Aa mutants in which a residue within these three loops was replaced with alanine revealed that the replacement of some residues in loop2 affected the mosquitocidal activity of Cry4Aa, but the effect was limited. This suggested that the receptor-binding site of Cry4Aa, unlike the well-characterized Cry1, was different from loops 1, 2, and 3. Although the contradiction in the necessity of loop2 observed as above should be solved, the fact that amino acid sequences in loops 1, 2 and 3 of Cry4Aa can be engineered without loosing toxicity suggests that these loops are an excellent target for modification to enhance the toxicity as well as to broaden the insecticidal spectrum of Cry4Aa. In this presentation, we also discuss about biological function of another loops α8, β4-β5 and β8-β9 of Cry4Aa.

Keywords: *Bacillus thuringiensis*, Cry4Aa

Contributed Paper Monday, 15:30 23

Description of the "ping pong" binding mechanism of Bt Cry1Ab toxin with *Manduca sexta* receptors

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Bacillus thuringiesis Cry toxins are used worldwide as insecticides in agriculture, forestry and in the control of disease transmission vectors. In the lepidopteran *Manduca sexta* cadherin (Bt-R_1) and aminopeptidase-N (APN) function as Cry1A toxin-receptors. The interaction with Bt-R_1 promotes cleavage of amino-terminal end including helix a-1 and formation of pre-pore oligomer that binds to APN, leading to membrane insertion and pore-formation. Loops of domain II and of domain III b16 of Cry1Ab toxin are involved in receptor interaction. Interaction with both receptors depends on the oligomeric state of the toxin. We characterized mutants in the Cry1Ab binding regions with respect to their binding to both receptors in both monomeric and oligomeric structures. Our results show that domain II loop 2 and loop 3 mediate different steps of Cry1Ab interaction with GPI-anchored receptors depending on their oligomeric state. These results suggests that APN fulfills two roles in the mode of action of Cry1Ab, first as a low affinity and highly abundant binding site that locates monomeric toxin in the vicinity of the microvilli before cadherin binding, and as a secondary high affinity receptor that mediates insertion of the oligomeric Cry1Ab structure into the membrane suggesting a ping pong binding mechanism. Also that the Cry1Ab binding epitopes in domain II loops and domain III undergo subtle structural changes upon oligomerization that are important for the sequential interaction with BtR_1 and APN receptors.

Keywords: Receptor binding, oligomerization, alcaline phosphatase, aminopeptidase, cadherin

Contributed Paper Monday, 15:45 24

Cyt1Aa synergizes the larvicidal activity of Cry2Aa against *Culex quinquefasciatus* through direct intermolecular interaction

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The Cyt1Aa toxin of *Bacillus thuringiensis* susbp. *israelensis* is known to synergize the mosquito larvicidal activity of the Cry proteins of this species, namely Cry11Aa, Cry4A and Cry4B, and delays the phenotypic evolution of resistance to these in *Culex quinquefasciatus*. More recently, it was shown that Cyt1Aa, which is highly lipophilic, synergizes

Cry11Aa by functioning as a surrogate membrane-bound receptor for this protein. However, synergy between Cyt1Aa and other Cry proteins is not known. Here we show synergy against *C. quinquefasciatus* between Cyt1Aa and Cry2Aa, a protein that has dual toxicity to certain lepidopteran and dipteran species. At the LC_{50} level, the degree of synergy using 1:1 ratios of two proteins was 4.1 for Cry2Aa + Cyt1Aa and 3.7 for *B. sphaericus* binary toxin (Bin) + Cry2Aa. A much higher level of synergy was obtained with a 1:1:1 combination of Bin + Cry2Aa + Cyt1Aa. Against larvae resistant to Cry4A + Cry4B, synergy was less than 2. To determine the mode of interaction between Cyt1Aa and Cry2Aa, we demonstrated by ligand binding assays that these toxins form complexes *in vitro*. Moreover, both toxins co-localized *in vivo* in the midgut of *C. quinquefasciatus* larvae. As Cry2Aa and Cry11Aa share structural similarity in Domain II, it is likely that the synergy between Cry2Aa and Cyt1Aa results from a similar mechanism previously proposed for Cry11A and Cyt1Aa. Our results suggest Cry2Aa might be a useful component for mosquitocidal endotoxin cassettes being developed for recombinant strains of *B. thuringiensis* subsp. *israelensis* and *B. sphaericus*.

Keywords: *Bacillus thuringiensis*, *Bacillus sphaericus*, Cyt1A, Cry2A, Bin

14:00-16:00 **View Posters**
Diseases of Beneficial Invertebrates, Microbial Control, Nematodes, Microsporidia,
(Authors stand by posters)

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

Symposium

Monday, 16:30-18:30
Hasan Turan

Biological Control of the Corn Rootworm (Diabrotica)

Organizer: Ken Narva

Symposium Monday, 16:30 25

The western corn rootworm in Europe: Current status and future challenges

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Current status of WCR spread in Europe

The western corn rootworm (WCR - *Diabrotica virgifera virgifera* LeConte; Coleoptera: Chrysomelidae), is one of the most destructive pest species in maize production systems. Larvae feed on the roots of maize plants, whereas adults may cause damage due to feeding on the silk. Originally known from North America since the fifties of the last century, this beetle invaded the South-Eastern parts of Europe in the late 80's. Since then WCR spread to several other countries in Europe, sometimes continuously extending its range by 50 km or more per year, and sometimes colonizing maize fields nearby airports far away from the front edge of the invasion. In general parameters contributing to the rate and pattern of spread of WCR in Europe are not investigated in detail. Molecular studies revealed that since the first detection of a WCR population in Europe four additional independent introduction events have taken place from North America to Europe in the past 20 years. Several hypotheses have been proposed to explain the recent multiple invasion events. Most reasonably, introductions and spreading of WCR in Europe have been paralleled in the US by an extension of the continuously colonized area to the Eastern parts of the US in the late decades of the last century, explaining to some extend the recent multiple introductions to Europe.

Given the establishment of this pest in Europe, on 22 October 2003 the EU defined emergency measures to prevent the spread of WCR within the EU member states. According to this decision member states need to conduct yearly official surveys for the presence of the insect in maize growing areas of their territory. In case of a detection of a single beetle previously not colonized by WCR, defined measures have to be initiated aiming at eradicating the insect. In 2006 this decision was amended to introduce additional requirements for the containment of WCR in infested zones and their vicinity, to limit the spread of the insect from the infested zones into areas that are free of the insect. Farmers with WCR adults caught in their fields are obliged to eradicate maize plants immediately and have to apply soil insecticides, whereas maize growers in nearby fields have to rotate corn at least once within 3 consecutive years. Because these strict eradication and containment measures are not appreciated by the farmers being concerned this directive entailed several studies as to evaluate the costs and benefits of these measures. These recently published economic assessments revealed substantial differences with regard to the production systems in different EU member states, depending for example on the proportion of continuous maize in the area or the extend of irrigation. Several economic assessment models are currently discussed, indicating that the control costs with regard to specific scenarios might exceed the benefits of the preventive containment and eradication measures required by the EU. However, these potential control costs will set the scene for evaluating different control measures, such as crop rotation, use of transgenic crops and breeding for resistance in conventional maize cultivars, chemical control, and biological control options.

Crop rotation, chemical and transgenic control options

Maize growers in the US have been coping with this pest in the past 60 years mainly by rotating corn with soybean and by insecticide applications. However, WCR has developed a resistance to various chemical insecticides (partly also found in the European populations), and, in addition, a specific variant of WCR currently found in the major maize growing regions of the corn belt, has developed a behavioural adaptation by laying eggs in soybeans (and to some extend also in other neighbouring crops). This allows development of the larvae, when these fields are rotated to maize the following season. Soil insecticides, which have been widely used in the US, will not have a prominent future in the EU, due to the political demand for an environmental friendly maize production, aiming at reducing the impact of these compounds on soil ecosystems. The development of transgenic crops expressing an insecticidal toxin from the bacterium *Bacillus thuringiensis* have expanded the control options for US farmers, whereas in most

European countries, due to public concern, this option for WCR control will not available within the forthcoming years.
The idea that WCR feeding will contribute to the problem of mycotoxin contamination of maize, as reported from plants infested with European corn borer larvae, has been tested recently. WCR may serve as a vector for Fusarium spores, and larval feeding contributes to a higher infestation incidence, given a high inoculum density of Fusarium is prevailing in the soil. Most scenarios tested with regard to contamination of maize plants by mycotoxins will not become the major problem in maize cultivation in Europe.

WCR alternative host plants and resistance breeding
Recently published surveys, conducted in the US and Europe have reported several grass species as potential alternative host plants for WCR development. The most crucial point in this respect is however, the amount of root biomass accumulated to sustain larval feeding and the synchronous occurrence of larvae and fresh root material in the field. In most cases, given the European climatic scenario, larval development and build up of root biomass will be met only under specific conditions. The potential of *Miscanthus x giganteus* cultivars, recommended and cultivated as a biomass crop has been evaluated recently both in the US and Europe. The specific agronomic and economic qualities of Miscanthus are a lack of susceptibility to pests, favouring its cultivation as a biomass crop. However, WCR larvae on Miscanthus developed as well as those grown on maize, allowing this grass to act as a refuge or reservoir for WCR populations in absence of maize cultivation.

WCR biological control options
In contrast to the US situation, biological control options targeting WCR have been extensively evaluated in several projects. One options related to the use of entomopathogenic nematodes (EPNs) will be presented in a separate presentation. There is a recent finding that maize roots, attacked by WCR larvae, emit a specific volatile attractive for entomopathogenic nematodes. Field data from the US provide evidence that EPNs may serve as an additional measure for WCR control in an integrated pest management approach. Plant breeders need to collaborate to develop maize cultivars with highly specific volatile signals interacting with the nematodes to meet economical feasibility by reducing applications costs of EPNs. The classical biological control approach by introducing a specific natural enemy of WCR from its area of origin to Europe needed to be evaluated further. A Tachinid fly (*Celatoria compressa*) which has been extensively investigated in this respect, and which proofed to have a narrow host range in Europe, restricted to the subtribes Diabroticina (New World) and Aulacophorina (Old World) within the tribe Luperini of the subfamily Galerucinae could regarded as a candidate for release in Europe. Enhancing the impact of natural enemies for the control of WCRT populations seems not a feasible strategy. A recently published laboratory and field study has shown that the predator complex of WCR is restricted, most probably due to a haemolymph defence strategy adopted by the larvae when attacked. The efficacy of entomopathogenic fungi (EPFs) in infesting WCR larvae have been tested in field experiments. The commonly used strategy by applying high doses of spores to the soil may be complemented by the recent findings that several EPFs may colonise the plant tissue endophytically and may then be ingested by the larvae feeding on these plant structures. These recent findings offer a fascinating new research avenue, allowing to provide maize plants with an integrative control mechanisms from the seeds onwards.

In conclusion, the development of control strategies for WCR in Europe will proceed in a more diverse way as compared to the US, by using cultural, chemical, biological, and resistance breeding options. Because the pest has not yet been able to colonise all high density maize growing areas in Europe, there is still time to develop and refine these diversified control strategies within the next years.

Symposium Monday, 17:00 26

Control of Western Corn Rootworms with the entomopathogenic nematode *Heterorhabditis bacteriophora*

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The chrysomelid beetle Western Corn Rootworm (WCR) (*Diabrotica virgifera virgifera* LeConte) invaded Europe in 1992 and has become a major pest in maize. The larvae are highly susceptible to the entomopathogenic nematode *Heterorhabditis bacteriophora* Poinar. Result of field plot experiments in southern Hungary and Austria between 2004 and 2009 will be presented and discussed. Different application techniques and timing of applications have been evaluated. Control assessed as number of emerging adults per plant ranged between 70 and 90 %. Damage to maize roots was significantly reduced. Maize hybrids emitting the nematode attractant beta-caryophyllene from their roots upon root feeding by WCR larvae facilitated the orientation of nematodes towards the pest larvae.

Keywords: Corn rootworm, maize, invasive pest

Symposium Monday, 17:30 27

Challenges to resistance management for transgenic maize targeting the western corn rootworm

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The western corn rootworm, *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) poses significant challenges the sustainable use of novel control strategies such as transgenic maize plants expressing *Bacillus thuringiensis* toxins. This insect has previously displayed a remarkable capacity for resistance evolution to both chemical and cultural control tactics that are uniformly deployed over large areas. Transgenic Bt plants targeting rootworms have been commercially available since 2003. However, unlike previous Bt maize events that target

lepidopteran pests, the efficacy of the rootworm targeted Bt maize is not "high dose" and the appropriateness of a resistance management strategies designed for high-dose events is uncertain. Moreover, recent laboratory selection experiments involving continuous exposure to transgenic roots throughout larval development and forced mating of survivors can cause increased survivorship and increased injury potential to transgenic plants in as few as three generations. Resistance management efforts are further challenged by the lack of adequate artificial diets and bioassay techniques that can be used for resistance monitoring and detection. A number of efforts are currently under way to develop improved resistance management approaches for rootworm targeted Bt maize events. An ncreased understanding of rootworm movement both as larvae and adults is developing and will assist in design and placement of appropriate refuges. Resistance monitoring efforts have yet to identify resistance among field populations but may lack the precision and sensitivity to reliably detect resistance should it occur, and continued efforts to refine resistance detection techniques are necessary. Second generation transgenic maize that produces two different Bt toxins has recently become available, but the precise target sites for these toxins have yet to be identified and the potential for cross resistance remains uncertain. Finally, novel transgenic approaches to rootworm control such as RNA interference (RNAi) are being developed. The availability of multiple and independent control options is likely to be an important component of the long-term sustainability of transgenic options for rootworm management.

Keywords: Transgenic, Bt maize, rootworm, resistance

Symposium Monday, 18:00 28

Assessing the impact of Corn Rootworm-resistant Bt maize on non-target predatory arthropods in laboratory and field studies

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The western corn rootworm, *Diabrotica virgifera virgifera* LeConte, is a serious maize pest and currently invading Europe. One strategy for its control is the use of genetically engineered maize expressing a Chrysomelidae-specific Cry protein derived from *Bacillus thuringiensis* Berliner. Most notably, plants expressing the Cry3Bb1 protein have already been grown on substantial *hectareages* in the United States.
Genetically engineered plants need to undergo an environmental risk assessment prior to their commercialization. One important part of this process is the non-target organisms safety assessment, with special emphasis on species providing important ecosystem services, such as biological control, pollination or decomposition.
This paper gives an overview of published studies on the potential impacts of Cry3Bb1-expressing Bt maize on non-target arthropods serving different functions. Most studies were conducted in the laboratory or glasshouse and unanimously demonstrate no negative effects of Cry3Bb1-expressing Bt maize or purified Cry3Bb1 protein on the non-target species investigated. In the field, no detrimental impact of rootworm-resistant Bt maize was reported.
To illustrate non-target risk assessment, we present data on the generalist predator *Theridion impressum* L. Koch. The spider was found to be exposed to Bt protein, after first analyzing its prey spectrum and measuring the Cry3Bb1 concentrations in potential prey species. Feeding studies with Bt maize fed prey and pollen did not indicate adverse effects. A field study aimed at assessing adverse impacts of Cry3Bb1-expressing maize on potential prey species also showed no negative impact. In contrast, conventional maize varieties were shown to substantially differ in terms of prey species densities. We conclude that Bt maize expressing Cry3Bb1 poses a negligible risk for this spider.
Our work and the published literature indicate that Cry3Bb1-expressing Bt maize is generally compatible with biological control for sustainable control of *D. v. virgifera*. The combination of laboratory and field studies in environmental risk assessment can be very informative. It was recognized, however, that field studies have a number of limitations which are discussed on the basis of some illustrative examples.

Keywords: Bt-maize, Diabrotica, biological

Contributed Papers
Monday, 16:30-18:30
Fahri Kuran

VIRUSES 2

Chairs: David Theilmann and Remziye Nalcacioglu

Contributed Paper Monday, 16:30 29

The *Autographa californica* multiple nucleopolyhedrovirus occlusion-derived virus envelope protein ODV-E56 is required for oral infectivity but is not essential for virus binding and fusion

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The *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) *odv-e56* gene encodes an occlusion-derived virus (ODV)-specific envelope protein, ODV-E56. To determine the role of ODV-E56 in oral infectivity, we produced recombinant EGFP-expressing AcMNPV clones (Ac69GFP-e56lacZ and AcIEGFP-e56lacZ) in which ODV-E56 protein synthesis had been eliminated by insertion of a beta-galactosidase expression cassette into the *odv-e56* open reading frame in either orientation. The *odv-e56* recombinant viruses exhibited no obvious alterations in polyhedra production and morphogenesis or in the production of infectious budded virus in cell culture. In bioassays using three lepidopteran host species (*Heliothis virescens*, *Helicoverpa zea*, and *Ostrinia nubilalis*), the oral infectivities of the *odv-e56* mutant viruses Ac69GFP-e56lacZ and AcIEGFP-e56lacZ were

profoundly impaired compared to those of wild type and control recombinant viruses. Oral infectivity against all three species was fully restored by marker-rescue of the *odv-e56* mutant viruses with either the AcMNPV or the *Rachiplusia ou* MNPV *odv-e56* gene. An *in vivo* fluorescence dequenching assay for virus binding and fusion indicated that *odv-e56* mutant viruses bound and fused to midgut epithelial cells at the same level as wild-type virus. Fluorescence microscopy of infected midguts, however, indicated that *odv-e56* negative viruses were unable to productively infect midgut cells. These results suggest that ODV-E56 is required for viral infection at a step following binding and fusion of the virion to the midgut epithelium.

Keywords: Baculovirus, AcMNPV, envelope protein

Contributed Paper **Monday, 16:45** **30-STU**

Baculovirus *per os* infectivity factors form a complex on the surface of occlusion derived virus

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Five highly conserved *per os* infectivity factors PIF1, PIF2, PIF3, PIF4 and P74, were reported to be essential for oral infectivity of baculovirus occlusion derived virus (ODV). Three of these proteins, P74, PIF1 and PIF2, have been reported to function in virus binding to insect midgut cells. Here evidence was provided that PIF1, PIF2 and PIF3 form a very stable complex on the surface of ODV particles of the baculovirus *Autographa californica* (Ac) MNPV. The complex could stand 2% SDS, 5% β-mercaptoethanol and heating at 50°C for 5 min. The complex was not formed when any of the genes for PIF1, PIF2 or PIF3 was deleted, while repair of these genes restored the complex. Co-immunoprecipitation (CoIP) analysis independently confirmed the interactions of the three PIF proteins and revealed that P74 is also associated with this complex.EM analysis showed that PIF1 and PIF2 are localized on the surface of ODV with a uniform distribution. This distribution does not change when the gene for the other PIF protein is deleted. We propose that these four proteins form an evolutionary conserved complex on the ODV surface, which has an essential function in the initial stages of virus infection.

Keywords: Baculovirus, ODV, *per os* infectivity factor, entry complex

Contributed Paper **Monday, 17:00** **31**

A peptide that binds the gut epithelium of *Heliothis virescens* has similarity to ODV-E66 and impedes infection with wild type baculovirus

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Lepidopteran nucleopolyhedroviruses (NPV) of the family Baculoviridae infect their host species via the midgut epithelium through binding and fusion of the occlusion-derived virus (ODV). The mechanism of ODV binding and fusion to the midgut epithelial cells is unknown. We screened a phage display library against brush border membrane vesicles (BBMV) derived from fourth instar *Heliothis virescens* midgut epithelia to identify gut binding peptides. We isolated two phage clones expressing gut binding peptides that had similarity to ODV-E66 from six species of alphabaculoviruses. ODV-E66 localizes to the ODV envelope and is conserved across lepidopteran baculoviruses. Chemically synthesized versions of these two peptides HV1 and HV2, and their homologs from *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV: AcE66-A and AcE66-B) bound to unfixed cryosections of whole midgut tissues. Binding of the peptides to *H. virescens* gut proteins was confirmed by far-western blotting with BBMV-derived proteins. Competition assays with HV1 and purified AcMNPV ODV resulted in decreased mortality at an LD50 dose, and a significant increase in survival time at lethal concentrations of virus. These results suggest that HV1 competes with AcMNPV ODV for binding to the *H. virescens* gut epithelial cells. The screening of phage display libraries for host tissue-binding peptides represents a novel approach for delineation of viral proteins and specific protein domains that function in host infection.

Keywords: Baculovirus, ODV

Contributed Paper **Monday, 17:15** **32**

Extensive proteomics analyses of the occlusion-derived virion of *Helicoverpa armigera* Nucleopolyhedrovirus

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In this study, the protein composition and localization of the occlusion-derived virion (ODV) of *Helicoverpa armigera* nucleopolyhedrovirus (HearNPV) were identified by multiple techniques. Advanced shotgun proteomics techniques including LC-Q-TOF MS/MS identified 43 proteins of HearNPV ODV, more than the 23 proteins we reported before. Quantitative mass spectrum analysis of the protein locations by using iTRAQ (isobaric tags for relative and absolute quantification) has successfully differentiated 18 envelope proteins and 13 nucleocapsid proteins. Western-blot and immuno-electron microscopy were used to confirm the protein localizations. In addition to the 31 proteins, Western-blot and immuno-electron microscopy analyses verified polyhedrin in the envelope as well as P78/83 and VP80 in the nucleocapsid fraction. In combination with previous reported data on ODV protein interactions, a model of ODV structure was schematically presented. This study is expected would promote further proteomic studies on baculoviruses, and would strengthen our understanding on BV/ODV assembly and infection mechanisms.

Keywords: Proteomics, ODV, HearNPV

Contributed Paper Monday, 17:30 33

Proteomic analysis of the occlusion-derived *Amsacta moorei* entomopoxvirus

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Entomopoxviruses are insect poxviruses that produce two types of infectious particles: the extracellular virus (ECV) and the occlusion-derived virus (ODV). In this study, we report the protein composition of the *Amsacta moorei* entomopoxvirus (AMEV) ODV. Purified ODV proteins were separated on denaturing gels and subjected to band excision, in-gel trypsin digestion and mass spectrometry (LC-MS/MS) using translated proteins of the AMEV genome and all available insect proteins as the reference data base. The number of proteins associated with AMEV ODV ranged from 100 – 144. Of these, 48 were orthologs of vaccinia virus (VACV) proteins most of which have been reported to be virion components. Orthologs of several membrane proteins which have been shown to assist in VACV entry as well as the formation of the viral entry-fusion complex were found indicating that entomopoxviruses employ similar mechanisms for gaining entry into host cells. Some of the core proteins that play roles in VACV structure and morphogenesis were also found to be components of AMEV ODV. Other proteins present in the occluded virus were orthologs of vaccinia proteins involved in transcription and DNA replication. A number of proteins associated with the ODV were found to be conserved in other viruses or other organisms. However, the majority were unknown proteins whose role in AMEV infection is yet to be determined. Consistent with most reports on other insect occlusion-derived viruses, no host proteins were found in AMEV ODV.

Keywords: AMEV, entomopoxvirus, proteomics

Contributed Paper Monday, 17:45 34

Deletion and functionally analysis of Group I Alphabaculovirus ODV specific genes and replacement with Grop II homologues

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There are two distinct genetic lineages in the Alphabaculoviruses that have been called Group I and Group II viruses. The genetic content of each group has diverged significantly but many genes are homologous. However it is unknown if homologous Group II genes can functionally replace Group I homologs or if their function is host or virus specific. In this study we have investigated the function of 17 genes shared between Group I and II viruses that were shown to be associated with Occlusion Derived Virions (ODV). The two viruses studied were *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) a group I alphabaculovirus and *Mamestra configurata* NPV-A (MacoNPV-A) a Group II virus. Using the AcMNPV bacmid system each gene was deleted and the deletion virus was subsequently repaired with the MacoNPV-A homolog or the WT AcMNPV gene, both tagged with the HA epitope. The majority of deleted genes severely impacted virus replication. For example, deletion of nucleocapsid encoding genes such as*vp39* and *vp80* abolished Budded Virus (BV) production. In addition, *gp41*, *p6.9*and *ac102* deletion viruses did not produce infectious viruses. In contrast deletion of *vlf-1* and *alk-exo*, resulted in infectious BV, although the yields were significantly reduced. Deletion of the replication genes associated with ODV abolished BV production. All of the deletion viruses were repaired by the MacoNPV-A homologues but most were unable to rescue any function. The exceptions included *Maco106* for *vlf-1*, *Maco82* for *vp80*, and *Maco84* for *ac102*. These results suggest that the majority of ODV genes have evolved to become virus or host specifi

Keywords: AcMNPV, MacoNPV baculovirus

Contributed Paper Monday, 18:00 35

The putative pocket protein binding site of *Autographa californica* Nucleopolyhedrovirus BV/ODV-C42 is required for virus-induced nuclear actin polymerization

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Nuclear filamentous actin (F-actin) is essential for nucleocapsid morphogenesis of lepidopteran nucleopolyhedroviruses. Previously, we had demonstrated *Autographa californica* multiple nucleopolyhedrovirus (*Ac*MNPV) BV/ODV-C42 (C42) is involved in nuclear actin polymerization by recruiting P78/83, an *Ac*MNPV *orf9* encoded N-WASP homology protein that is capable of activating an actin-related-protein 2/3 (Arp2/3) complex to initiate actin polymerization, in the nucleus. To further investigate the role of C42 in virus-induced actin polymerization, a recombinant bacmid vAc$^{p78/83nls\text{-}gfp}$, featured with *c42* knockout and *p78/83* tagged with a nuclear localization signal coding sequence and an *egfp* as a reporter gene under the control of *Pp10* promoter was constructed and transfected to Sf9 cells. In the nuclei of vAc$^{p78/83nls\text{-}gfp}$ transfected cells, polymerized F-actin filaments were absent, whereas other actin polymerization elements (i.e. P78/83, G-actin, and Arp2/3 complex) were present. This *in vivo* evidence indicated that C42 is actively participating in the nuclear actin polymerization process as a key element, besides its role in recruiting P78/83 to the nucleus. In order to collect *in vitro* evidence for C42 participating in actin polymerization, anti-C42

antibody was used to neutralize the viral nucleocapsid which is capable of initiating actin polymerization *in vitro*. Both pyrene-actin polymerization kinetics and F-actin specific staining by phalloidin indicated that anti-C42 can significantly attenuate F-actin formation efficiency as compared with control antibodies. Furthermore, we have identified the putative pocket protein binding sequence (PPBS) on C42 that is essential for C42 to exert its function in nuclear actin polymerization.

Keywords: AcMNPV, BV/ODV-C42, nuclear actin polymerization

Contributed Paper **Monday, 18:15 36**

Autographa californica Multiple nucleopolyhedrovirus Ac92 (ORF92, P33) is required for budded virus production and multiple-envelope-occlusion-derived virus formation

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In this study, the *Autographa californica* M nucleopolyhedrovirus (Ac*M*NPV) *orf92* (*ac92*, *p33*), *ac92*, one of the 30 genes present in all sequenced baculovirus genomes, was characterized. Transcription analysis by RT-PCR showed that *ac92* transcripts initiate from a typical ATAAG late-transcription start motif, starting at 9 h post infection. The production of Ac92 protein was detected from 24 h post infection in Ac*M*NPV-infected Sf9 cells. Ac92 was associated with the envelope of both budded virus and occlusion-derived virus (ODV) and with the nucleocapsid of ODV. To investigate the role of Ac92 during the virus replication cycle, an *ac92* knockout bacmid was generated through homologous recombination in *Escherichia coli*. Titration and plaque assays showed a lack of virus spread in *ac92*-knockout-virus-transfected Sf9 cells. Quantitative real-time PCR analysis and immunoblottings demonstrated that the *ac92* deletion did not affect viral DNA synthesis or viral protein synthesis. Electron microscopy showed that in the *ac92*-knockout virus transfected cells, multiple-nucleocapsid-ODV was not observed; instead, single-nucleocapsid-ODVs were detected in the ring zone related to the intranuclear microvesicles. To learn more about the function of Ac92, an Ac92 mutant virus was made in which the $C^{155}XXC^{158}$ amino acids were mutated to $A^{155}XXA^{158}$. Viral replication and electron microscopy analysis showed that the mutant virus has the same characterizations as the knockout virus. Ac92 wild-type and mutant proteins were expressed, purified and characterized for enzymatic activity and results showed that the mutant protein lost the sulfhydryl oxidase activity. These data suggest that the C-X-X-C motif is essential for the function of Ac92.

Keywords: AcMNPV, Ac92, P33

Contributed papers
Monday, 16:30-18:30
Nihat Turan 1

BACTERIA 2

Chairs: Juan Ferre and Sabahat K. Ozman Sullivan

Contributed Paper **Monday, 16:30 37**

Comparison of midgut proteolytic activity in susceptible and induced-resistant populations of *Plutella xylostella* to *Bacillus thuringiensis* subsp. *kurstaki* 3a3b

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A field collected strain of the diamondback moth, *Plutella xylostella* L. showed high susceptibility to a Bt product based on *Bacillus thuringiensis* subsp. *kurstaki* 3a3b. A resistant colony was created following six continuous generations in laboratory. We estimated LC_{50} for both populations in laboratory and greenhouse tests. One of the resistance mechanisms in caterpillars involves gut proteases that interact with *Bacillus thuringiensis* toxins. Based on previous studies, Serine proteases predominantly trypsin-like are important in proteolytic processes in lepidopteran larval guts. Therefore, the midgut protease activities of the *B. thuringiensis* induced-resistant and susceptible populations of the diamondback moth were assayed on substrates HEMOGLUBIN 2% and on BApNA for total and tryptic activities, respectively. Six hours after feeding 4th instar larvae of both populations on Bt treated and untreated canola leaves, midgut of these larvae was isolated. These midguts were determined as 12 group-assays including whole midgut, midgut wall and midgut contents in each substrate and each population. Following related protocols, free peptides through the activity of proteinases on HEMOGLUBIN and BApNA were recorded using microplate reader at 630 and 405nm, respectively. Control (Blank) was also considered with adding TCA to reaction mix before adding enzymatic extract. Assays were carried out with three replicates. Data analysis indicated that there are significant differences for tryptic activity on BApNA and also for total proteolytic activity on Hemoglubin in three different midgut areas between susceptible and resistant populations fed on Bt treated leaves but these differences were not significant for larvae fed on healthy canola leaves between these two populations. These results which supported the role of Diamondback moth's proteolytic system in development of resistance to Bt, will be discussed in details.

Keywords: Diamondback moth, resistance, *Bacillus thuringiensis*, proteolytic activity, midgut

Contributed Paper **Monday, 16:45 38**

Molecular characterization of *Bacillus thuringiensis* using REP-PCR (Repetitive Element Polymorphism)

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An element of repetitive DNA sequences has been studied in prokaryotes bodies. These sequences are highly conserved and is present in multiple copies in the genome of most Gram-negative bacteria and several Gram-positive and can be used to produce "fingerprints". The genetic characterization by repetitive and conserved sequences in bacterial genomes, especially sequences of REP (Repetitive Extragenic Palindromic) and ERIC (Enterobacterial Repetitive Intergenic Consensus) has been performed by polymerase chain reaction (PCR).This tool has the advantage of using known primers and submit sequences that have been used in studies with several species of bacteria. The present study aimed to estimate the genetic divergence among 56 strains of *Bacillus thuringiensis* based on the ERIC and REP sequences, their group, and possible relationships of these groups with the subspecies and larval mortality in *Spodoptera frugiperda*. The fragments generated were analyzed by electrophoresis in agorose or polyacrylamide gels, depending on the primers. The genetic distances were obtained by addition of the coefficient of Jaccard, and groups were performed by the UPGMA method with application of bootstrap to check the consistency of the groupings. The dendrograms generated by ERIC and REP primers were not informative when evaluated separately, however when analyzed together, it showed great genetic diversity among 56 strains of *B. thuringiensis*, with formation of 21 groups when considered a distance of 60% as a cutoff point. The formation of groups appears to be related to mortality and origin, requiring further study for confirmation.

Keywords: Repetitive sequences, *Bacillus thuringiensis*, genetic distance

Contributed Paper **Monday, 17:00 39**

Inheritance of Cry1Ac resistance in cotton bollworm, *Helicoverpa armigera* Hubner, and its implications in resistance management in Bt cotton

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Transgenic Bt cotton was grown over 15 million hectares in 2009 in the world, of which 8.4 million hectares (about 85% of cotton area) alone was grown in India, thereby constituting an ever-increasing selection pressure on target lepidopterans especially, cotton bollworm, *Helicoverpa armigera*. Bt resistance management that includes non-Bt cotton or Pigeon pea as refuge crops is mandated on the assumption of inheritance of Cry1Ac resistance being a recessive trait. However, resistance in *H. armigera* to Cry1Ac is found semi-dominant/semi-recessive to dominant in nature and hence, the refuge crop may not serve the purpose of producing progeny susceptible to Cry1Ac as expected. Further, number of genes involved in Cry1Ac resistance varies depending up on resistance levels attained by the target pest under selection pressure. The increasing levels of tolerance of *H. armigera* to Cry1Ac have been a trend since Bt cotton introduction in 2002 until date in India. Possibly, fitness costs associated with resistance evolution in *H. armigera* may aid in delaying resistance and sustaining Bt cotton. The complexity of Bt resistance management is also due to non-compliance of Bt resistance management practices and development of various events like those of variants of Cry1Ac and dual stacked Cry1Ac and Cry2Ab2 in cotton with varying levels of expression of toxins. The durable Bt resistance management must focus on the use of stacking of different Bt genes for delaying evolution of resistance and providing broad spectrum of insecticidal activity, and phasing out single stacked transgenics within broad aegis of integrated pest management.

Keywords: Insect resistance, *Helicoverpa armigera*, inheritance, Bt cotton

Contributed Paper **Monday, 17:15 40**

Characterization of binary toxin from *Bacillus sphaericus* ISPC-8

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Bacillus sphaericus is an aerobic, endospore forming gram positive bacterium having toxicity against different mosquito species. In our laboratory different mosquitocidal strains of *B. sphaericus* were isolated. Among the strains, local isolate ISPC-8 was the most viable and virulent isolate exhibiting significantly higher total viability count and lower LC_{50} values compared to standard strains 2362 and 1593. This isolate contains gene encoding Mtx and binary toxin proteins BinA and BinB. The binary toxin from this isolate was purified from the inclusion bodies and the purified toxin exhibited LC_{50} dose of 6.32 ng/ml against *Culex quinquefasciatus* larvae. The binary toxin genes (*bin*A and *bin*B) were cloned and expressed using *E. coli* expression system. Interestingly, the recombinant BinA protein alone showed significant toxicity with LC_{50} dose of 66.9 ng/ml against *C. quinquefasciatus*. Akin to the wild-type proteins, the equimolar concentration of *in-situ* folded recombinant BinA/BinB exhibited very high toxicity with LC_{50} dose of 7.86 ng/ml against *C. quinquefasciatus*. Interestingly, the BinA protein from ISPC-8 differ by one amino acid (R197M), while BinB differs by two amino acids (H99P, P174S) as compared with 1593 and 2362 strains.

Keywords: *Bacillus sphaericus*, purification of binary protein, *Culex quinquefasciatus*

Contributed Paper Monday, 17:30 41

Characterization of plasmid patterns of *Bacillus thuringiensis* efficient against *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae)

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Bacillus thuringiensis Berliner harbors genes encoding Cry proteins found in chromosomes or plasmids of different sizes (4-150 MDa). Although the smaller plasmids are more abundant in B. thuringiensis, their specific function isn't known. As for the megaplasmids, their main recognized function is harboring cry genes, although the sequencing of some of these plasmids indicates the occurrence of other important genes. This work used a new protocol for practical and rapid extraction of plasmid DNA, in order to characterize the plasmid patterns of strains belonging to Embrapa Maize and Sorghum Research Center *B. thuringiensis* bank, in Brazil. We tried to further assess the relationship of the plasmid patterns with strains belonging to the same serovars. Results showed that it was possible to characterize 49 out of the 59 strains based on the migration of bands in agarose gel. Strains belonging to the same serovars showed different plasmid size, with the exception of two strains belonging to serovars *galleriare*. The strain T09 *Bt tolworthi* showed identical plasmid migration such as strains belonging to serovar *galleriare*. Plasmid patterns were different for 46 strains, confirming that this technique is a useful tool to discriminate against specific strains.

Keywords: Cry protein, *Bacillus thuringiensis*, plasmid, megaplasmids

Contributed Paper Monday, 17:45STU 42

A new Sugar phosphate sensor system in *B. cereus* induced during oral infection of *Galleria mellonella*

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Bacillus thuringiensis and *B. cereus* are closely related gram positivebacteria and pathogens for insect larvae. In order to identify genes specifically expressed during infection an *in vivo* expression technology (IVET), system was developed in *B. cereus* and tested in *Galleria mellonella* larvae, 20 genes were identified [I]. Here we focus on one which is part of a five-gene cluster consisting of a two-component system (TCS): *spsR* and *spsK*, and three downstream genes designated as *spsA*, *spsB*, and *spsC*. Specific *spsABC* transcriptional (*gus/gfp* fusions) promoter activation was localized in the midgut and no activity was found in LB medium. However, following testing several midgut related substrates, glucose-6-phosphate (G-6-P) was found to stimulate transcription in LB. This transcriptional activity was abolished in the *spsRK*, *spsA* and *spsB* mutants while strong expression was found in the *spsC* mutant. Interestingly, similar expression behaviors were found with *gfp* fusions analyzed during infection of *G. mellonella*. This indicates that the *spsABC* transcription is activated by SpsRK in response to G-6-P stimuli, and that the *spsA spsB* and s*psC* genes are essential for the activation/function of this new sugar signal pathway. Further investigations related to virulence and the full understanding on regulation and role of all components are ongoing.

[1] Fedhila, S., Daou, N., Lereclus, D., Nielsen-LeRoux, C., 2006. Identification of *Bacillus cereus* internalin and other candidate virulence genes specifically induced during oral infection in insects. Mol. Microbiol. 62(2), 339-55

Keywords: B. cereus,Galleria mellonella,Sugar phosphate sensor system,Glucose-6-phosphate

Contributed Paper Monday, 18:00 43

Molecular characterization and insecticidal activity of a highly pathogenic isolate of *Bacillus thuringiensis* from Adana

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In this study we have determined a higly pathogenic *Bacillus thuringiensis* isolate (SY49.1) on some insect pests from Adana and this isolate were analyzed to study the presence of cry genes.

By using general and specific primers we have determined that this isolate harbor cryIC, cryIAa, cryIB, cry5, cry9A, and cry9C genes. This isolate might carry some other cry genes which are not determined in this study. Plasmid profile of SY49.1 isolate was different from some standard strains. The number of plasmid bands of SY49.1, Bt *kurstaki*, Bt *israelensis*, and Bt *tenebrionis* were 9, 6, 8, and 4, respectively. Crystal protein profile of this isolate was considered to range between 25 and 140 kDa. The SY49.1 isolate was shown to have bipyramidal, spherical and cuboidal crystal proteins in different sizes in Transmission Electron Microscope imaging. The larvicidal activity of this isolate was tested against some important lepidopterean pests at the doses ranging from 250 to 7000 ppm. The SY49.1 isolate showed complete mortality on *Ephestia kuehniella*, *Plodia interpunctella* and *Thaumetopoae pityocampa* larvae at all the doses tested. The LD50 and LD99 values of this isolate were 5 to 6 times lower for E. kuehniella and 6 to 10 times for P. interpunctella compared to standart strain Btk HD1. The nucleotide sequence of PCR products amplified by 16S-ITS rDNA primer showed 98% similarity with *Bacillus thuringiensis* serovar *andalousiensis* BGSC 4AW1 in megablast analysis. Further molecular characterization

is needed to elucidate the highly pathogenic nature of this isolate. After safety assessments, this isolate could be very promising candidate for formulations and commercial applications to control wide range of economically important pest insects.

Keywords: *Bacillus thuringiensis*, SY49.1, *Ephestia kuehniella*, *Plodia interpunctella*, *Thaumetopoae pityocampa*, cry gene

Contributed Paper Monday, 18:15 44

Cheap genomes: Identification and classification of spontaneous mutations by genome sequencing

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Genome sequences are generated for a fraction of a penny per base, opening the floodgate for rapid sequencing of any bacterial genome. Using comparative genomics, these data permit the characterization of every genomic change occurring among any set of related bacteria. Although the costs for generating genomic sequence continue to fall, costs associated with assembling, annotating, and analyzing this deluge of data have not. CoGe, a publicly available web-based software system, creates a suite of interconnected analytical and visualization tools for rapidly comparing genomes from all domains of life. We've extended CoGe's tools for whole genome mutation and polymorphism identification to analyze the genomes of 8 derivative clones of a robust wild-type *Escherica coli* K12 strain, NCM3722. We contracted the sequencing of these genomes to Roche and their sequences arrived with *de novo* contig-level assemblies and no annotations -- a typical state for newly minted genomes. We developed a novel method to create a pseudomolecule assembly of these genomes using the best syntenic path generated by comparison to the fully sequenced reference strain, *E. coli* MG1655. Initial analysis identified ~1800 putative polymorphisms, many of which were false positives – too many to validate using conventional cloning and resequencing techniques. Using CoGe, we identified three major classes of false positives – low sequencing fold-coverage, homopolymer sequencing errors and mis-assembly of local tandem repeat sequences. These permitted us to remove low-coverage genome sequences and develop a false positive scoring scheme to rank and prioritize polymophisms thus reducing our putative polymorphism table to 120 low-scoring polymorphisms. Of these, 35 were either previously known differences among the sequenced strains or newly validated, representing a 29% true-positive hit rate. Together, this reusable analytical pipeline creates a comparative genomic map for quickly identifying mutations arising in these derivative strains, and the overlying affected genes. Of general interest, these methods and tools are applicable to all bacterial genomes, including the 18 publically available sequenced strains of *Bacillus thuringiensis*.

Keywords: Cheap genomes, pseudomolecule

Monday, 18:30-20:30
KTU SAHIL

DINNER

Monday, 20:30-22:30

SIP DIVISION BUSINESS MEETINGS AND WORKSHOP

Fungi Nihat Turan1
Nematode Nihat Turan2
Bacteria Hasan Turan

Virus Fahri Kuran

Business Meeting

Workshop: Bioinformatics

Organizer: Eric Haas-Stapleton
The dynamic structure and evolution of genomes
Eric Lyons, UC Berkeley, US

Microsporidia Preparation room

Business Meeting

Workshop: Genomics of microsporidia

Small, beautiful and sexy? Insight from a decade of microsporidian genomics.
Joe Ironside, Aberysytwyth University, Aberysytwyth, GB

22:45 Buses return to hotels

POSTERS
Diseases of Benefical Invertebrates, Microbial Control, Microsporidia, Nematodes.

DISEASES OF BENEFICAL INVERTEBRATES

Poster / Benefical Invertebrates Monday,14:00
DBI-01

Demonstrating effective RNAi products to manage factors of Honey Bee colony collapse

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The Colony Collapse Disorder (CCD) phenomenon is still not fully understood or agreed upon; however, there is a strong consensus some specific pathogens and pests are major contributing factors to Colony losses. Viruses in general and Israeli Acute Paralysis Virus in particular, microsporedia such as the Nosema Cerana and the Varroa mite are considered the top three contributors to the phenomena. Beeologics has developed a generic technology platform which is utilized to introduce a full RNAi product-line targeting all three pathogenic groups:Developing Remebee™ required acquiring know-how in all aspects of RNAi product introduction including: designing RNAi control strategies, proof of concept, implementation of large scale field trails, regulation, and economically effective large scale production. In this presentation will provide the main methodology and proofs in our goal to control the major pests associated with honey

bee colony losses. The development of generic RNAi platform capabilities and the potential RNAi strategy represents opened a wide spectrum of potential collaborative work with scientists working on invertebrates health and control.

Keywords: RNAi, honeybee, virus, nosema, varroa, targeted pest control

Poster / Benefical InvertebratesMonday, 14:00
DBI-02

Multiplex PCR detection of slowly-evolving trypanosomatids and neogregarines in bumblebees using broad-range primers

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Aims: The aims of this study were to design universal markers for different protozoan parasites of *Bombus* spp. based on the phylogenetic position of two important bumblebee parasites *Crithidia bombi* and *Apicystis bombi.*
Methods and Results: Standard PCR and extraction techniques were used to amplify and sequence 18S rDNA. Phylogenetic analysis of the rDNA was performed in order to predict the parasite-range of the primers.
Conclusion: *C. bombi* phylogenetically clusters with the trypanosomatids with slowly-evolving SSU-rRNA sequences (SE), while *A. bombi* is the closest sister group of *Mattesia*. A multiplex was designed containing an internal control and two broad-range primer pairs, detecting *C. bombi* and other SE trypanosomatids and also *A. bombi* and other neogregarines.
Significance and Impact of Study: Sequence data generated will further improve the current systematics of insect trypanosomatids and gregarines which remain troublesome. Broad-range markers for bumblebee parasites are necessary tools enabling the screening of commercially imported colonies and thus controlling their worldwide distribution and to discover related emerging parasites.

Keywords: Protozoa detection, multiplex PCR, phylogeny, *Crithidia bombi*, *Apicystis bombi*, 18S rDNA

Poster / Benefical Invertebrates Monday, 14:00
DBI-03

Recent research on *Galleria mellonella* as one from most important insect model organisms

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The greater wax moth *G. mellonella* (*Gm*) L. (Lepidoptera: Pyralidae) is one from most widely used insects in physiology, immunology, biochemistry and parasitology; even when is considered as a pest for the apiculture. Surprisingly this classical model was not sequenced yet, so can't compete with *Drosophila* or *Tribolium* in this area. The advantages of *Gm* are fast lifecycle, size of larvae, easy rearing on artificial diet and commercializing in countries like: Spain, Germany, United States, Mexico, Argentina, Chile and Peru. We review the most recent (2007-2010) published articles using *Gm*. Many reports determines growing degree day for egg, larvae and pupae which similarly to the proteins and carbohydrates levels in *Gm* adults varied in relation to light photoperiod. *Gm* in the toxicology bioassays has been recently used to test chemical insecticides, parasitoides, nematodes, fungical, bacterial and microorganism metabolites etc. applied by injection, ingestion or direct contact. Other reports used *Gm* larvae to study changes in antioxidant enzymes and oxidative stress after application of insecticides (boric acid, sodium tetraborate), inhibitors of eicosanoids, antibiotics or infection with *B. thuringiensis* resulting to impaired enzymatic antioxidant defense capacity and metabolic functions with increasing oxidative stress. *Gm* is widely used to test the pathogenity of nematodes with potential application to biological pest control, newly also co-infections are studied. This review of *Gm* publications shows importance of insect research in pest control with impact in agriculture and also with possible extrapolation to medicine.

Keywords: *Galleria mellonella*

Poster / Benefical Invertebrates Monday, 14:00
DBI-04

Report of Coccinellid and orius species of corn fields in Isfahan region

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In a study of corn field in Isfahan region, Althogether 7 species of Coccinellids (Coccinellidae: Coleoptera) and 5 Species of Orius bugs (Anthocoridae: Hemiptera) were collected and identified as follows:
A- Coccinellidae:
1- *Coccinella septempunctata*
2- *Coccinella undecimpunctata*
3- *Hippodamia variegata*
4- *Oenopia conglobata*
5- *Propylea quatuordecimpunctata*
6- *Hyperaspis syriaca*
7- *Nephus sp.*
B- Anthocoridae:
1- *Orius albedipennis*
2- *O. niger niger*
3-*O. niger aegyptaicus*
4- *O. vicinus*
5- *O. horvath*

Keywords: Occinellid, Orius, corn, Isfahan

Poster / Benefical Invertebrates Monday, 14:00
DBI-05

Determining Microfungus Flora of Body Surface and Intestinal System of Caucasian Race Bees (*Apis mellifera caucasica* Pollmann, 1889) (Hymenoptera: Apidae)

Mehmet Ali Kırpık[1]; **Mehmet Nuri Aydogan[2]**; **Serkan Örtucu[2]**; **İsmet Hasenekoglu[2]**;

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Honey bees are economically important species to Turkey and many other regions of the world due to honey production and also vital pollinators for many species. Therefore, extensive microfloral honey bee research conducted in the literature. Early research was focused on the isolation of pathogen, however contemporary efforts are devoted to unveil ecological role of the non-pathogen members of flora and their potential ecological benefits. In the present study, we evaluated microfungus flora of digestion system and body surface of dead and alive Caucasian (*Apis mellifera caucasica* Pollmann, 1889) race bees collected from Kars plateau in 2009. We identified 13 different species of fungi belonging Penicillium, Alternaria, Mucor, Trichoderma, Fusarium, Aspergillus, Ulocladium, Verticillium, and Zythia genuses. The relationship between these fungi and honey bees was discussed further in the light of the existing literature.

Keywords: *Apis mellifera caucasica*, Caucasian Honey Bee, Intestine System, Microfungus

MICROBIAL CONTROL

Poster / Microbial Control Monday, 14:00 MC-01

Reduction of pesticides pollution by microbial control of soil-borne pests: A case study

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In developing countries, agricultural pest control mostly relies on chemical control and health hazards and environmental pollution have become a serious problem. Microbial pest control including use of entomopathogenic fungi (EPF) is an important component of ecologically based pest management programs. In this study we investigated agricultural soils of Shahrood region, north east of Iran, for presence of EPF. The result showed that more than 78% of soil samples had EPF. This indicates potential of indigenous EPF for microbial control of soil-borne pests. Potato tuber moth (PTM) is a serious pest and several chemical insecticides are used to control its damage. Six strain of EPF isolated from soil samples were tested on this pest. Results indicated that all isolates tested have potential to control of this pest. This survey have shown that agricultural soils even in dry climates are rich of EPF and therefore we can use them for the control of soil-born pests while protecting biodiversity in the agroecosystems and avoiding toxic hazards of insecticides to human and environment .

Keywords: Pesticides Pollution, microbial control, entomopathogenic fungi

Poster / Microbial Control Monday, 14:00 MC-02

Perspectives for determining bioactivity of three commercial Lepidopteran microbial insecticides based on *Bacillus thuringiensis*

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The bioactivities of commercial *Bacillus thuringiensis* (Bt.) based microbial insecticidal products are unpredictable and are often difficult to compare and to quantify. Difficulties arise when product brands use different approaches to determine and publicize potency claims on product labels. Methods most commonly used for estimating product bio-potency or product bioactivity are toxin assay by HPLC, SDS-PAGE and (or) Insect Bioassay. Each of these methods has its own advantages and disadvantages. The objective of this presentation is to compare reliability of these methods in determining the bioactivity of three commonly sold commercial Bt. products. The pros and cons of these methods are evaluated with discussion on their reliability in predicting the "true" bioactivity of Bt. based products.

Keywords: *Bacillus thuringiensis*, HPLC, biopotency, bioassay

Poster / Microbial Control Monday, 14:00 MC-03

The physiological characterization and functions of poly-β-hydroxybutyrate (PHB) in *Bacillus thuringiensis*

Chen Deju[1]; He Jin[1]; Zhang Qingye[1]; Li Mingshun[1]; Jiang Heng[1]; Sun Ming[1]; Yu Ziniu[1]
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Poly-β-hydroxybutyrates (PHBs) are accumulated during exponential growth and then utilized very fastly during the early stationary phase in *Bacullus thuringiensis*. PHB is an important reserve substance as carbon and energy resource. However, it is still little known on how the PHB affects those physiological functions. Here we constructed two stains: *Bacullus thuringiensis* PHB accumulation defected strain PHB$^-$ (the *phaC* was knocked out in BMB171) carrying *cry1Ac* (PHB$^-$cry) and a control strain, BMB171 carrying *cry1Ac* (171-cry). A comparison proteomics of the two strains revealed that many proteins, such as Pgi , GapA and AtoB were down-regulated. While CitZ CitB and aldehyde dehydrogenase were up-regulated, at exponential phase in PHB$^-$-cry compared to 171-cry. Those genes expression changed resulted in excreting more acetate, pyruvate and citric acid than those of the control. We found that proteins SpoIIQ and SpoIVA were down-regulated at early stationary in the mutant, and the mRNA of *sigF*, *sigK*, *spo0A* and *kinA*, obtained from RT-PCR analysis, were down-regulated in mutant compared to the control. So, we can conclude that the accumulated PHB is important to *Bacillus thuringiensis* significantly forms spore, and synthesizes crystal insecticide protein.

Keywords: *Bacillus thuringiensis*, poly-β-hydroxybutyrates (PHBs), physiological characters, functions

Poster / Microbial Control Monday, 14:00 MC-04

Monitoring on the resistance of Diamondback Moth to *Bacillus thuringiensis*(Bt) engineering strain WG-001

Congchong Zhang[1]; **Yizhe Wang[1]**; **Zhe Zhang[1]**; **Jianglin Yao[1]**; **Ziniu Yu[2]**; **Jianhong Li[1]**
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In this paper, susceptibility of *Plutella xylostella* collected from different areas in central China to *Bacillus thuringiensis*(Bt) Engineering Strain WG-001 was monitored using leaf-dipping technique. The results showed that *Plutella xylostella* collected from four areas produced resistance to WG-001. The field populations of diamondback moth from Yueyang and Wuxue produced moderate level resistance with the resistance index (RI) 14.20 and 10.48, respectively, the population collected from Yichang produced low level resistance with the resistance index 5.77**,** and susceptibility of the population collected from Luoyang was susceptible to WG-001 with resistance index 2.92.

Keywords: Resistance, Diamondback moth, *Bacillus thuringiensis*

Poster / Microbial Control Monday, 14:00 MC-05

Characterization of culturable bacteria from *Ostrinia nubilalis* (Lepidoptera: Crambidae) and their insecticidal effects on the pest

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European corn borer (ECB) (*Ostrinia nubilalis* Hbn. Lepidoptera: Crambidae) is a polyphage pest and widespread in all over the world. ECB larvae cause a serious damage to maize as well as millet, hemp, hop, and it is capable of injuring peppers, sorghum, cowpea, soy-bean, fruit of beans and cotton. Studying the bacterial flora of the pests are important for both determining a significant bio-control agent and insect resistance. For this purpose, *Ostrinia nubilalis* larvae were collected from various fields of Eastern Black Sea region of Turkey. Total of 26 different cultured bacteria were isolated from these larvae and identified as *Pseudomonas aeruginosa* (On1), *Brevundimonas aurantiaca* (On2), *Chryseobacterium formosense* (On3), *Acinetobacter* sp. (On4), *Microbacterium thalassium* (On5), *Bacillus megaterium* (On6), *Serratia* sp. (On7), *Ochrobactrum* sp. (On8), *Variovorax paradoxus* (On9), *Corynebacterium glutamicum* (On10), *Serratia* sp.(On11), *Paenibacillus amylolyticus* (On12), *Microbacterium* sp. (On13), *Alcaligenes faecalis* (On14), *Microbacterium testaceum* (On15), *Enterococcus* sp. (On16), *Enterococcus* sp. (On17), *Leucobacter* sp. (On18), *Paenibacillus* sp. (On19), *Alcaligenes faecalis* (On20), *Microbacterium* sp. (On21), *Microbacterium* sp. (On22), *Leucobacter* sp. (On23), *Alcaligenes faecalis* (On24), *Serratia marcescens* (On25), *Serratia* sp. (On26). Our results indicates that isolates coded as On13, On16, On19 and On22 are probably novel isolates. The highest insecticidal effect was 100% by On1 (*Pseudomonas aeruginosa*) and On16 (*Enterococcus* sp.) againts the 3th instar pest larvae within fourteen days. Consequently, On1and On16 appear to be the most promising bio-control agent against *Ostrinia nubilalis*.

Keywords: *Ostrinia nubilalis*, Bacterial flora, Insecticidal activity, Microbial control

Poster / Microbial Control Monday, 14:00 MC-06

Native isolates of fungal pathogens to control aphids in Uruguay

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In Uruguay several aphid species damage forage grasses and legumes by direct feeding with probability to toxin and virus transmission. Climatic change and high input agriculture increase population abundance and alter existing balances between phytophagus insects and natural control agents. Fungi belonging to the entomopathogenic genera: *Metarhizium* spp., *Beauveria* spp., *Paecilomyces* spp. and *Lecanicillium* spp. were isolated from Uruguayan insect pests. Strains of these genera were applied in spore suspensions ($1.0x10^7$ conidia/ml approximately) with or without a basic formulation, glycerol (01% w/v) and gum xhantam (0.1% w/v), against pasture damaging greenbug *Schizaphis graminum* (Rondani). Aphid colonies were maintained on oat plants growing on substrate within glass flasks. Number of alates and their offspring were counted before treatments and 4, 7, 10 and 13 days after. Pathogenicity for all genera ranged between 21.4 and 68.7% in spore suspensions without formulation. Best control for alates, 73.7% and 52.6%, was obtained with *Lecanicillium* spp. and *Beauveria* spp., respectively, in spore suspensions with formulation. Only *Paecilomyces* spp. and *Lecanicillium* spp. showed pathogenicity against nymphs, 26.4 and 39.4%, respectively. When considering both aphid stages only *Paecilomyeces* spp and *Beauveria* spp showed good performance, 64.3% and 55.6%, respectively. *Lecanicillium* spp. in suspensions with formulation gave the highest adult mortality, 73.7%. In this work a narrow strain selection was obtained based on the pathogenicity results. Formulation improved the performance in the *Metarhizium* strains and maintained the susceptibility toward the different fungi strains in alate aphids. For aphid nymphs formulation did not improve pathogenicity

Keywords: *Schizaphis graminum*, *Paecilomyces* spp, *Lecanicillium* spp, *Beauveria* spp, strain selection

Poster / Microbial Control Monday, 14:00 MC-07

Microbiological control of the red palm weevil *rhynchophorus ferrugineus* with *Beauveria bassiana* and *Metarhizium anisopliae*

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The red palm weevil (RPW) *Rhynchophorus ferrugineus* Olivier is a very severe threat to ornamental palms in Italy. Strategies for control of the larval and adult stages of the insect include the use of virulent strains of entomopathogenic fungi. In 2009 and 2010 we carried out laboratory trials to evaluate the efficacy of local strains of *Beauveria bassiana* and *Metarhizium anisopliae* isolated from naturally infected RPW adults feeding on palms in central and southern Italy.
Treatments were carried out via direct contact of individuals with fungal cultures; the insects were rolled on the cultures and then kept in small plastic containers in a controlled environment at 27°C and 45-60% relative humidity.
At 28 days after larval treatment, the *B. bassiana* strain had caused around 50% mortality and the *M. anisopliae* strain 60 and 100% mortality. In adult trials, 20 and 30% of the weevils were killed by *B. bassiana* and 53 and 85% by *M. anisopliae*. In the control trials, the respective values were 13% and 20% for larvae and 7% and 10% for adults.
The results show good control of larvae and adults, suggesting the possibility of application of the microbiological control strategies in the field. However, further research is required on the types of formulation and application of the fungal strains.
Paper produced within the ambit of the Research Project "*Difesa nei confronti del Punteruolo rosso delle palme, **Rhynchophorus ferrugineus** – DIPROPALM*", financed by MiPAAF - Ministero delle Politiche Agricole Alimentari e Forestali (D.M. 684/7303/08, 11/03/2008).

Keywords: *Rhynchophorus ferrugineus*, RPW, entomopathogenic fungi, *Beauveria bassiana*, *Metarhizium anisopliae*

Poster / Microbial Control Monday, 14:00 MC-08

Determination of cellular immunity of insect pests collected from Kahramanmaras province, Turkey due to natural microbial infections

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Naturally occurring entomopathogenic microbes are virulent insect pathogens. One of the important barriers to successful deployments of microbial control agents may lie in insects' robust and complex innate immune effectors. In the context of this work, these mechanisms also can limit the effectiveness of microbes deployed for biocontrol of insect pest populations. Because the potential for infection is high, we formed the hypothesis that most of the insects living in agrarian fields experience natural infections. Here we report on the outcome of a field investigation designed to test our hypothesis. Pest species representing Coleoptera, Lepidoptera, Hemiptera and Orthoptera were collected from fields surrounding Kahramanmaras, Turkey. Following identification, the specimens dissected under stereomicroscope to asses numbers of nodules present in the insect bodies. Nodulation was assessed in a total of 19 insect species collected during spring and summer of 2003-2005. In the broadest description, we recorded nodules in 98% of the 435 specimens examined, although there was a very wide range of nodules/specimen (from 1 nodule/insect to >100 nodules/insect). We recorded more nodules from insects found in soil, a site of significant microbial challenge, than other sites. The key implication of our finding is that robust immune effector systems limit the host range and effectiveness of microbial agents deployed for biological control. Future advances in the efficacy and use of biopesticides will depend on understanding and somehow attenuating the efficacy of insect innate immune effector systems. Some insect pathogens have already evolved effective mechanisms to achieve this advance.

Keywords: Cellular Immunity, Microbial Infections

Poster / Microbial Control Monday, 14:00 MC-09

Entomopathogenic fungi for locust control in the Republic of Georgia, a multifaceted evaluation

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Orthoptera, esp. *Caliptamus italicus* and *Dociostaurus maroccanus*, represent frequent, severe sources of crop loss in the Caucuses, esp. Georgia. Thus there is need to identify indigenous entomopathogenic fungi for locust control as alternatives to chemical pesticides. As part of a program to build insect pathology infrastructure within in Georgia, a program was conducted to identify potentially useful fungi for locust control. Five isolates of *Beauveria bassiana* and eight isolates of *Metarhizium anisopliae* were made from Georgian Orthoptera. These isolates were evaluated in terms of conidial production in a pilot-scale, solid-substrate fermentation system; vegetative growth under constant temperatures of 10, 15, 20, 25, 30 35 and 37° C. and also transient 39 and 41° C., mimicking periods of behavioral fever in host insects; and bioassay efficacy against *Melanoplus sanguinipes* and/or *Schistocerca americana*. Six of eight *Metarhizium* isolates produced >40 g conidia/kg substrate. All *B. bassiana* isolates were very poor conidia producers, tending towards extensive mycelial growth. There was considerable variability among the isolates in their tolerance to temperatures <15° C. and >30° C., as measured by radial growth on agar media, as well as to 3-9 hr transient exposure to 39 or 41° C. Many showed a 1-2 day lag before resuming normal growth upon return to 28° C. All of the isolates were highly virulent for either of the two acridids. Several of the *M. anisopliae* isolates have potential for operational use as mycoinsecticides against Georgian Orthoptera. This research was funded by US Agency for International Development grant TA-MOU-03-CA23-022.

Keywords: Orthoptera, Metarhizium, Beauveria

Poster / Microbial Control Monday, 14:00 MC-10

Initiation, characterization and karyotyping of a new cell line from Red Palm weevil *Rhynchophorus ferrugineus* adapted at 27°C

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Insect cells have been successfully cultured in vitro as continuous cell lines for over 35 years. The media, culture methodology and conditions have been well resolved such that, for many insects, new cells lines can be routinely developed. One of the major rationales for developing insect cell lines was for the study of insect viruses and other (natural or chemical) agents impact or mechanism. This was particularly true for species of Lepidoptera from which over 900 viruses have been reported (Dwight E. Lynn 1999). Since many species of *Coleoptera* are serious agricultural and palm pests specially the Red Palm Weevil (*Rhynchophorus ferrugineus*) regarding the difficulties and obscurities in field study of these pests, cell culture initiation and rearing systems were established, effects have been made to utilize some of these pathogens as biological pesticides. Cell cultures are important in this endeavor since viruses require a living cell. Primary cultures were initiated from pupae ovaries and another from Embryos of *R. ferrugineus* which were reared in the laboratory in GEBRI for several generations as describe by Steve H, *et al* (2002) with some modification as described by Murhammer (2007). The medium employed in this study to initiate primary cultures was modified Grace's medium supplemented with 20% inactivated (56°C/30 min) fetal bovine serum. The cell cultures were observed daily using a phase contrast inverted microscopy at 400x magnification and morphologically characterized were as spherical at the beginning then most of them turned to be spindle shaped. Profiles of *Rhynchophorus ferrugineus* cultures *in vivo* and *in vitro* were characterized by using the RAPD PCR, isozymes, protein profile and karyotyping(work still on going to finish cell cloning and characterization by flowcytometer). Treatments by using three types of viruses were carried out, results analysis and viral identification is on going.

Keywords: Cell cultures, *R. ferrugineus*, *in vivo*, *in vitro* characterization, RAPD PCR, isozymes, protein profile and karyotyping, viral infect and identification

Poster / Microbial Control Monday, 14:00 MC-11

Protection afforded by an emulsifiable oil against imbibitional damage in *Metarhizium anisopliae* conidia

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It was recently demonstrated that dried *M. anisopliae* (Ma) conidia formulated in pure oil are not severely impacted when subjected to a cold water-based substrate, whereas unformulated, dried conidia may have their viability dramatically reduced depending on imbibition temperature. So far, the protective role of emulsifiable oils (EO) against imbibitional damage (ID) has not been tested, despite their increasing importance in mycopesticide formulation. In this work, conidia of two isolates within the Ma complex were dried over silica gel for 3 days at 25 ºC, then mixed or not with an EO (Natur'l Oleo), and subsequently subjected to water (0.05% tween 80) held at 0, 25, or 37 ºC. A germination protocol adapted from Oliveira (2010) allowed EO removal from conidial suspensions through the use of a surfactant. Resulting suspensions were inoculated onto agar medium and incubated at 25 ºC for either 24 or 48h (in the later case, the medium was amended with a fungistatic). Dried Ma conidia plunged into cold water were severely damaged (0% germination at 24h p.i.), whereas those treated at 25 and 37 ºC showed viabilities of 36-47 and 79-84%, respectively. On the other hand, viabilities for dried EO-formulated conidia plunged into water at 0, 25 and 37 ºC were 7-12, 81-90, and 88-93%, respectively. In general, germination counts at 48h p.i. were slightly higher than counts performed at 24h p.i. (data not shown). These results underscore the importance of formulating ID-sensitive conidia.

Keywords: Microbial control, Dehydration, Formulation

Poster / Microbial Control Monday, 14:00 MC-12

The effects of host-plant resistance on the susceptibility of the diamondback moth to *Bacillus thuringiensis*

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Many field populations of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), a worldwide pest of cruciferous plants, has developed resistance to synthetic pesticides. In this regard, more sustainable technologies including biological control and plant resistance are needed urgently to control *P. xylostella*. Here, laboratory studies were performed to explore combined effects of resistant host-plantand *Bacillus thuringiensis* (*Bt*) on *P. xylostella* mortality. The cultures of *P. xylostella* were kept on different host plants, including *Brassica pekinensis* (Chinese cabbage) cv. Hero, *B. oleracea* var. *botrytis* (cauliflower) cv. Royal, and *B. oleracea* var. *capitata* (common cabbage) cv. Globe Master and cv. Ascara. These host plants are susceptible (Hero), intermediate (Royal and Globe Master) and partially resistant (Ascara) to attack by *P. xylostella*. The susceptibility of pest larvae was then tested using two preperations of *Bt kurstaki*. The results demonstrated that the susceptibility of *P. xylostella* to *Bt* was influenced by the host-plant resistance. Indeed, *Bt* acted better on pests fed on resistant host plants compared with that on susceptible host plants. The interaction of the pathogen and the plant resistance resulted in more mortality of the pest, implying a synergistic effect. From a pest management viewpoint, this may be

promising for integration of the pathogen and the partially-resistant host plants against *P. xylostella* in field studies.

Keywords: Host-plant resistance, Diamondback moth, *Plutella xylostella, Bacillus thuringiensis*

Poster / Microbial Control Monday, 14:00 MC-13

Entomopathogenic fungi sprayed at different times on *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs and parasitism by *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae)

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Trichogramma pretiosum is often used in association with entomopathogenic fungi (EF) for pest control. This study aimed to evaluate the effect of EF sprayed on *Anagasta kuehniella* eggs at different times (96, 72, 48, 24 and 0 hours) previously to parasitism by *T. pretiosum*. Five groups were prepared (20 card each with ± 200 eggs) and sprayed with *Beauveria bassiana* (*Bb*) (Unioeste 47 and Unioeste 57), *Metarhizium anisopliae* (*Ma*) (Unioeste 43 and Esalq 09) and *Isara fumosorosea* (*If*) (CB 369 and CB 394) and then submitted to parasitism. The same procedure was carried out with the to control. There was no difference in the number of eggs parasitized at different hours in the Control, *Bb* (Unioeste 57) and *If* (CB 369). However, the mean number of parasitized eggs per card (approximately 200 eggs per card) when sprayed with *Ma* (Unioeste 43) and *Bb* (Unioeste 47) was higher at 72 h (20.2 and 28.6 respectively) and lowest at 0 h (8.4 and 5,3 respectively). For *If* (CB 394) and *Ma* (Esalq 09) the largest number of parasitized eggs was at 0h (25.2 and 24.3), and lowest at 96 (6.4) and 72 h (6.2), respectively. When compared in the same hour, the parasitism in control was not different from that in eggs sprayed with the fungi. However, differences between the strains were observed. Thus, in a strategy for use of both BC agents the fungus strain and the time between spraying and parasitoid released should be taken into account.

Keywords: Microbial control, compatibility

Poster / Microbial Control Monday, 14:00 MC-14

Repellence of entomopahogenic fungi applied on *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs to the parasitism by *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae)

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In many crops *Trichogramma pretiosum* and entomopathogenic fungi are used simultaneously. However, there are no studies about the compatibility between these two biological control agents. So, the aim of this study was to evaluate the repellence effect of entomopathogenic fungi over *T. pretiosum* parasitism. Two strains of each entomopathogenic fungi *Beauveria bassiana* (Unioeste 47 and Unioeste 57), *Isaria fumosorosea* (CB 369 and CB 394) e *Metarhizium anisopliae* (Unioeste 43 and Esalq 09) were used. Two cards (1.0 x 5.0 cm) containing *Anagasta kuehniella* non parasitized sterilized eggs were fixed inside of tubes, and one of these cards was sprayed with a fungus suspension (1.0 x 10^9 conidia/mL) and the other sprayed with only water and Tween (control). Then, one female of *T. pretiosum* was released inside the tube. For each treatment (fungus/strain) 20 replications were made. The parasitism was evaluated and each strain was compared with the control using Wilcoxon test. It was observed that the strains promoted repellence of *T. presiosum* with exception of the strain CB 394 *I. fumosorosea* (53.08% treated and 46.92% non treated). Repellence of *T. pretiosum* from eggs treated with the fungi occurred for *B. bassiana* (Unioeste 47: 34.25%, Unioeste 57: 27.65%), *M. anisopliae* (Unioeste 43: 22.99, Esalq 09: 29.88) and *Isaria fumosorosea* (CB 369: 19.22%). Also, there were differences between strains. Probably this is due to the ability of *T. pretiosum* in identifying repellent substances on the surface of eggs. Therefore, in developing an IPM program using these BC agents their compatibility should be studied.

Keywords: Microbial control, Compatibility

Poster / Microbial Control Monday, 14:00 MC-15

Spread dynamics of *Hyphantria cunea* Drury in Georgia and its natural enemies

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Hyphantria cunea Drury - American Fall Webworm is an invasive pest insect in Georgia, which was firstly recorded in 70s of 20th century. After that periodically occurs rapid increase of its population number and harm to agricultural and forest plants as well.

From 2004-2005 outbreak of *H.cunea* started in Samegrelo region and gradually was spread in whole West Georgia. Investigation was carried out on dynamics of pest population in 2005-2009.

The following natural enemies - parasitoids and predators, which are tropically connected with *H. cunea* was studied in above mentioned years: 2 species of *Hymenoptera*, 2 species of *Diptera*,1 species of *Neuroptera*, 2 species of *Coleoptera*. In the conditions of Georgia they were noticed for the first time.

It was established that the most effective natural enemies are the pupa parasitoids *Psychophagus omnivorus* Walk. *(Hymenoptera: Pteromalidae)* and *Chouioia cunea* Yang. (*Hymenoptera: Eulophidae*). Investigations were carried out on *H.cunea* natural enemies spread dynamics also in above mentioned years in Samegrelo region, where pest was settled on almost every deciduous trees except of eucalyptus and

laurel. Spread of pest was reached to culmination in 2007, when about 7-11 nest was noticed in every deciduous tree.
Phenology details of *H.cunea* for the given region was studied. Amount in percentage of parasitized pupa during years was established. The role of described parasitoids on pest number regulation was determined.

Keywords: *Hyphantria cunea* Drury, natural enemy, parasitoid

Poster / Microbial Control Monday, 14:00 MC-16

Appearance of pathogens within outbreak populations of native insect populations in New Zealand

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The Gondwanaland remnant of Aotearoa-New Zealand developed a unique flora and fauna over >60 million years. When settlers introduced European grasses and clovers they initially grew spectacularly in the absence of their usual pest complex. However, some native insects were capable of adapting to the new resources and reached unprecedented numbers destroying the developing pastures. After a temporary respite from use of broad spectrum chemicals, an uneasy balance of nature has been reached across New Zealand grasslands, except where major new land conversions has caused fresh pest outbreaks. On the New Zealand West Coast, 'flipping" of swamps to create new pastures has reset the conditions to total environmental disruption. After initial vigorous pasture growth, a species previously unknown as a pest, *Pyronota feastiva* (Manuka beetle), has reached numbers exceeding 1000/m^2 within 5 years of land development. The enemy release hypothesis states that invasive animals and plants thrive in new environments due to the lack of natural enemies present in their home range. While this hypothesis has generally been investigated for exotic invasions around the world, invading organisms can also be indigenous, having moved into new or disrupted ecosystems. Research has indicated a growing awareness of the importance of pathogens in population regulation and points to the importance of specificity in the host pathogen relationship. However, specific evolved relationships cannot explain the rapid development of epizootics of disease in invasive pest populations. We have recently discovered infection by fungi and *Rickettsiella* in the Manuka beetle population. We will present and discuss our findings in relation to the appearance of microbial pathogens and their role in regulating new pest populations.

Keywords: *Pyronta* spp., Manuka beetle, pest outbreak, pasture, pathogen, fungi, *Metarhizium*, bacteria, Rickettsiella

Poster / Microbial Control Monday, 14:00 MC-17

The genome-scale metabolic network reconstruct and comparative analysis for *Bacillus thuringiensis* strain YBT-1520

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The *B. thuringiensis* strains YBT-1520, which is highly toxic to *Lepidopteran* pests and was isolated by our Lab and protectedby patent (Chinese patent number: ZL 95 106749.4). The preparetions of strains YBT-1520 are widely used for controlling pests of agriculture and forestry. *B. thringiensi*s 97-27 and *B. cereus* ATCC 14579 belong to *B. cereus* group too, but they can't produce ICPs. The prospect of understanding the relationship between the genome and the physiology of an organism is an important incentive to reconstruct metabolic networks. To better understanding the mechanism of ICP forming, we have reconstructed genome-scale metabolic model of YBT-1520, Bt97-27 and Bc14579 strains based on their recently genome, and physiological and biochemical data from the literature by using the AnEnPi software. By comparative analysis on the Carbohydrate Metabolism, Amino Acid Metabolism and Energy Metabolism network of the three strains respectively, important different points were obtained within their metabolism network. We have determined some important enzymes, which exist only in YBT-1520, and didn't correspondingly present in two other strains. The products catalyzed by these enzymes were all important for the ICP forming. To verify the predicted results, the validating experiments were performing on.

Keywords: *Bacillus thuringiensis*, *B. cereus* group, metabolic networks, comparative analysis

Poster / Microbial Control Monday, 14:00 MC18STU

Investigation of the mode of action of toxins *of Bacillus thuringiensis* Cry1Aa and Cry1Ac and study of their interactions with the intestinal receptors of *Ephestia kuehniella*

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Bacillus thuringiensis is characterized by the production of crystals composed of proteins with a highly specific insecticidal activity against larvae of several species of insects. The mechanism of action of these d-endotoxins involves: solubilization of the crystals in the midgut of the larva, proteolysis of the protoxin, attachment of the active toxins to the intestinal cells and finally their integration into the apical membrane by forming pores. A comparative study of different steps in the mode of action of the individual *B. thuringiensis kurstaki* BNS3 Cry1Aa and Cry1Ac d-endotoxins on *E. kuehniella* larvae was performed in order to investigate the origin of the difference in the response of this larva to each of the latters. The absence of two protease activity and the alteration of the proteolytic activation level of the midgut juice in the case of Cry1Aa could be a way of tolerance of the insect. *In situ* binding and

histopathological effects showed that Cry1Aa is slightly attached to the apical microvilli of epithelial cells that remained intact while Cry1Ac is strongly linked to the membranes of the damaged epithelial cells. Analysis of susceptibility to digestion by pronase of both free and bound toxins to *E. kuehniella* BBMVs and sequence alignment showed the presence of a difference of one amino acid at position 148 of a 4 helixes. This difference is most likely responsible of the difference in toxicity of these toxins toward *E. kuehniella*, and the amino acid F148 is probably a key one in the oligomerization step.

Keywords: *Bacillus thuringiensis*, *E. kuehniella*, protease activity, In situ binding, histopathological, pronase, oligomerization

Poster / Microbial Control Monday, 14:00
MC19STU

Microbial control of Diamondback Moth, *Plutella xylostella*, using Entomopathogenic fungus *Beauveria bassiana* and *Lecanicillium* spp.

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Effect of application using *Beauveria bassiana* and *Lecanicillium* spp. to *Plutella xylostella* in field were investigated for two years (2008~2009). Each conidial suspension (2.0×10^7/ml) of MG-Bb-1 (*B. bassiana*) and 2aF27 (*Lecanicillium* spp.) were splayed twice on cabbage foliage during growing stage. In 2008, the number of larvae and pupae in control plot increased high level (16.5~20.2 /plant) during 3 weeks after inoculation. Whereas in MG-Bb-1 and BT-formulation plots, larvae and pupae density was significantly low level (7.41 and 3.2 larvae and pupae/plant) in 3 weeks after inoculation. Same as in 2009, MG-Bb-1 and BT plots resulted in lower density of larvae and pupae (under 10.3 /plant) compared to control plot for 5 weeks after inoculation. The present study suggested that MG-Bb-1 could have the potential for microbial control of diamondback moth in Hokkaido Japan.

Keywords: Biological control, diamondback moth, *Plutella xylostella*, *Beauveria bassiana*, *Lecanicillium* spp.

MICROSPORIDIA

Poster / Microsporidia Monday, 14:00 M-01

Effect of *Vairimorpha ephestiae* (Microsporidia: Burenellidae) on detoxifying and antioxidant enzymes in *Galleria mellonella* larvae

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The effect of the microsporidian *Vairimorpha ephestiae* Mattes (Microsporidia: Burenellidae) on nonspecific esterases (NE), glutathione S-transferases (GST) and superoxide dismutases (SOD) was studied in tissues of the larvae of *Galleria mellonella* L. (Lepidoptera: Pyralidae). The increase of NE activity was registered in hemolymph of infected larvae during 15 days of experiment. An overexpression of esterase isozyme in hemolymph has been already detected at the 3rd day post infection. No changes in esterases pattern were observed in the fat body's homogenates of the *G. mellonella* larvae possessing the symptoms of microsporidiosis. The degradation of esterase isozymes and the decrease of NE activity in the pattern of the midgut homogenates of infected larvae were registered during parasite sporogony. The greatest NE activity in hemolymph and midgut tissues was registered during vegetative reproduction of parasite, but the least level of NE activity was observed during mass sporogony of microsporidia. GST activity in hemolymph of infected insects decreased at the stage of merogony, whereas during mass sporogony of parasite the enzymatic activity in host tissues was higher than in control. The results presented clearly showed that the levels of NE, SOD and GST correlated with developed microsporidia infection in *G. mellonella* larvae.

Keywords: microsporidia, nonspecific esterases, glutathione S-transferases, superoxide dismutases

Poster / Microsporidia Monday, 14:00 M-02

Intestine microsporidia of the genus *Liebermannia* from Argentine grasshoppers: morphology, taxonomy, life cycles and routes for speciation

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Three microsporidia species from the genus *Liebermannia* (Sokolova, Lange, Fuxa, 2005) parasitize different parts of alimentary tract of Argentine grasshoppers (Orthoptera): *L. patagonica* inhabits midgut epithelial cells of *Tristira magellanica* (Tristitidae), *L. dichroplusae* – Malpighian tubules of *Dichroplus elongates* (Melanoplidae), and *L. covasacrae* – salivary glands of *Covasacris pallidinota* (Acrididae). All three species produce small (2.5-3.0 X 1.1-1.5 um) ovacylindrical spores by sequentially splitting off sporoblasts. Sequence similarity of the SSU rDNA among three congeners is 97%-99%, and all three species fell into one clade in dichotomy with *Orthosomella-Endoreticulatus-Euplotospora* group, in SSU rDNA-based phylogenetic trees with 100% bootstrap support. *L. patagonica* is diplokaryotic all over the life cycle. *L. dichroplusae* produces elongated multinuclear merogonial and sporogonial plasmodia with diplokarya and monokaryotic spores. *L. covasacrae* displays similar life cycle, except the number of nuclei in sporonts is always two. Meronts of *L. patagonica* and *L. covasacrae*, but not *L. dicroplusae* are enveloped in host ER. *Liebermannia* spp. probably have evolved as specialized parasites of the alimentary

tract. *L. patagonica* adapted to parasitizing midgut epithelium, which demanded a shortened lifecycle to survive the rapid turnover of midgut epithelial cells. Wrapping the most vulnerable presporogonic stages in host cell ER may help to protect the parasite against the phago-lyzosome system. *L. dichrpolusae* and *L. covasacrae* subsequently might have evolved to infect Malpighian tubules and salivary glands with less antagonistic environments. These species developed their own specific characteristics but preserved a life cycle with meiosis followed by dihaplophase/haplophase transition.

Keywords: Microsporidia ultrastructure molecular phylogeny

Poster / Microsporidia Monday, 14:00 M-03

Subtraction analysis of *Nosema bombycis* infected IPLB-LD-652Y cell line

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Microsporidia are a group of parasites related to fungi that infect a wide variety of animals and have genomes that range in size from 2.3 to 19.5 Mbp. *Nosema bombycis* is the pathogen that causes epidemic pebrine disease in *Bombyx mori*. The cDNA of IPLB-LD652Y cells (LD cells) and the *N. bombycis* infected LD cells were hybridized and total 165 cDNA fragments were obtained after subtraction. The result could be divided into eight categories: genomic sequences, mitochondrion genome, ribosomal protein, 18S rRNA with poly-A tail, *elongation factor-1α*, *heat shock protein*, microsporidia specific genes and other genes. Most of these cDNA fragments were cloned from host cells. Only fourteen cDNA fragments (9%) are microsporidia specific genes, including ribosomal proteins, histone, retrotransposon Nbr7 and hypothetical proteins. Of these cDNA fragments, five cDNA fragments (3%) are the 18S rRNA with poly-A tail, this result revealed that such arrested translation is also caused by microsporidian infection, therefore, the occurrence of these fragments may not only be a possible consequence of picorna-like viral infection but also *Nosema* spp. infection. The 18S ribosomal fragments and presence of multiple viral infection and microsporidian infection may provide to be useful diagnostic markers for colonies afflicted with CCD.

Keywords: Microsporidia, *Nosema bombycis*, cDNA subtraction

Poster / Microsporidia Monday, 14:00 M-04

Effects of an ant species, *Formica fusca*, on the transmission of microsporidia infecting gypsy moth larvae

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Predators can influence transmission of a pathogen. If infected prey is not avoided, the predator could disseminate inoculum while preying and thus enhance horizontal transmission. But inoculum could also be removed from the system. Moreover, the predator could become infected. In the present study, we tested how predation by the forest ant *Formica fusca* interacts with *Nosema lymantriae* and *Vairimorpha disparis*, two microsporidian pathogens of the gypsy moth, *Lymantria dispar*, and how it affects transmission. When given a choice, *F. fusca* preferred gypsy moth larvae infected with *N. lymantriae* over uninfected larvae. On the other hand, they avoided *L. dispar* larvae infected with *V. disparis*. *F. fusca* never became infected with *N. lymantriae* or *V. disparis* after feeding on infected prey. No significant effects of predation by *F. fusca* ants on the transmission success of *N. lymantriae* and *V. disparis* were ascertained; when infected and uninfected susceptible test larvae were placed onto caged and potted oak plants for five or ten days no altered percent infection in test larvae was measured regardless if foraging ants were present or not. Observational studies in an oak forest showed that both, infected and uninfected *L. dispar* larvae placed at oak trees or on the ground attracted workers of *F. fusca*. While infected *L. dispar* cadavers were never attacked, the ants preyed on 11% of the uninfected and 25% of the infected larvae that were exposed.

Keywords: Microsporidia, Predation, Transmission, *Lymantria dispar*

Poster / Microsporidia Monday, 14:00 M-05

***Hepatospora eriocheiri* n.gn. n. sp. infecting Chinese mitten crabs (*Eriocheir sinensis*) and a proposal for erection of a new family (Basosporidae) to contain phylogenetically similar microsporidians from aquatic crustaceans**

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A new species of microsporidian parasite is described infecting the hepatopancreas of an invasive decapod crustacean species (*Eriochier sinensis*) from the River Thames, London, United Kingdom. Numerous stages in merogony and sporogony of the parasite were observed using histopathology and electron microscopy. Uninucleate meronts, appeared to exist within a simple unit membrane very closely opposed to a second membrane, lacking attached ribosomes but likely of host origin, within the cytoplasm of hepatopancreatic epithelial cells. Uninucleate meronts underwent nuclear fission to form bi- and multi-nucleate meront plasmodia that remain bound within a simple plasmalemma. Plasmotomy of multinucleate meront plasmodia involved the formation of elaborate membrane systems between sets of pre-sporont nuclei and the eventual separation of uni-, bi- and multinucleate sporonts within the vacuole. Individual sporonts developed a significantly thickened cell wall. Sporonts either developed directly to sporoblasts via generation of spore extrusion precursors (anchoring disk and polar filament) or underwent nuclear division and budding to form further sporonts. At this stage, multi-nucleate sporonts were apparently able to develop similar spore extrusion precursors to those observed in uninucleate sporont stages. Furthermore, bi-nucleate sporonts containing these precursors

appeared to undergo division to form uninucleate stages (these presumably progressing to mature spores). Multi-nucleate sporont plasmodia were also observed forming spore extrusion precursors though these appeared aberrant and are presumed not to progress to mature spores. Mature spores contained a polaroplast with an outer (compressed) and inner region (coiled) region, a terminal anchoring disk and 7-8 coils of an isofilar polar filament apparently contained within an additional membranous system. Ultrastructural observations of this parasite are somewhat consistent with members of the genus *Cystosporogenes* (Family Glugeidae) while its presence within a crustacean host and a distinct molecular phylogeny suggesting relatively closer affinity to members of the Enterocytozoonidae supports the formation of a new genus and species (*Hepatospora eriocheir*), and likely, a new family (Basosporidae), with relatively close affinity to the Enterocytozoonidae. Consideration of morphological and phylogenetic characteristics of other hepatopancreas-infecting microsporidians from crustaceans suggests that these may also be re-classified into this new family. *Hepatospora eriocheiri* is the type species of the type genus within this new family. Based upon morphological and host similarities, we also propose reclassification of *Endoreticulatus eriocheir* from Chinese mitten crabs from China as the same pathogen to that reported here from invasive Chinese mitten crabs in the United Kingdom.

Keywords: Microsporidia, ccrustacean, mitten crabs

Poster / Microsporidia Monday, 14:00 M-06

Occurrence of *Nosema oryzaephili* in *Cryptolestes ferrugineus* and transfer to the genus *Paranosema*

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A microsporidium that closely resembles *Paranosema* species at the level of the light microscope was isolated from the rusty grain beetle, *Cryptolestes ferrugineus*. It's identity as *N. oryzaephili* (originally described from *Orzaephilus surinamensis*) was confirmed by comparison with a known isolate of *N. oryzaephili* based on spore size, small subunit rDNA sequence, and relative infectivity to *Oryzaephilus surinamensis*, *Tribolium castaneum*, and *Ephestia kuhniella*, Phylogenetic analysis of the small subunit rDNA indicates clearly that this species belongs in the genus *Paranosema* and thus the designation *Paranosema oryzaephili* (Burges, Canning and Hurst) is proposed. In spite of the abundance, economic importance, and world-wide distribution of *C. ferrugineus*, this is the first report of a microsporidial infection in this species. This is also the first report of *P. oryzaephili* in the new world.

Keywords: Microsporidia, Coleoptera, Paranosema, grain beetle

Poster / Microsporidia Monday, 14:00 M-07

Light microscopic and molecular detection of microsporidia infecting *Loxostege sticticalis* (Lepidoptera: Pyraustidae) in Eurasia

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Presence of microsporidian infections was estimated using light microscopy (LM) and polymerase chain reaction (PCR) in two populations of the beet webworm *Loxostege sticticalis* collected in the European part of Russia in 2007 (N=25) and 2008 (N=36) as well as in one archive specimen of a dried larva, heavily infected with microsporidia, collected in Eastern Siberia in 1982. LM of fresh smears accompanied with DAPI staining revealed infection with diplokaryotic spores in both European samplings at incidence rates of 4% (2007) and 2.8% (2008). Screening the same datasets using PCR with V1f:530r and V1f:1492r universal microsporidia primers resulted in incidence rates of 8% (2007) and 5.6% (2008), thus suggesting higher sensitivity of this diagnostic tool as compared to LM observation of the fresh smears. Ribosomal DNA sequence analysis suggested allocation of the microsporidian haplotype to the genus of *Endoreticulatus.* The archive specimen of 1982 showed presence of multiple octospores, and PCR was positive only with V1f:530r primer set. Sequencing of the PCR product identified this haplotype as a member of the *Nosema/Vairimorpha* clade. The research is supported by RFBR nos 10-04-00284 and 09-04-00619 and a grant from RF President no MK-3419.2009.4.

Keywords: microsporidia, beet webworm, rDNA, molecular phylogeny

Poster / Microsporidia Monday, 14:00 M-08

Morphological and molecular variability in the *Nosema-Vairimorpha* species complex infecting *Lymantria dispar*

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The *Nosema-Vairimorpha* microsporidian complex that infects the gypsy moth, *Lymantria dispar*, forms a species group distinct from other taxa in the *Nosema-Vairimorpha* clade. Morphology and interaction with the host justified previous descriptions of several isolates from *L. dispar* as different species and divided the group into two genera placed in different families, Nosematidae and Burinellidae. Molecular and proteomic analyses, however, show much closer relationships, certainly at the generic level and possibly at the species level. rDNA and HSP-70 gene sequences are identical (>99% identity) for all *L. dispar Nosema* species and *Vairimorpha disparis.* A

few substitutions and polymorphic sites occur in the SSUrDNA among the geographic isolates of *Nosema lymantriae*. Our studies, including ultrastructure, gene sequences, protein profiles, and bioassays, provide information on the biological variation within this genetically related group.

Keywords: Microsporidia, Nosema, Vairimorpha, genetic identity, species complex, biological variations

Poster / Microsporidia Monday, 14:00 M-09

Transmission of a microsporidium from the convergent lady beetle, *Hippodamia convergens*, to the green lacewing, *Chrysoperla carnea* Stephens

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Convergent lady beetles, *Hippodamia convergens* Guérin-Méneville, and green lacewings, *Chrysoperla carnea* Stephens, are two common natural enemies that are used throughout North America for aphid biological control. This study examined the transmission potential of a microsporidium in *H. convergens* to *C. carnea* and the effects of the pathogen on host fitness. Uninfected and microsporidia-infected *H. convergens* eggs were fed to uninfected lacewing larvae. Pathogen transmission to *C. carnea* was 7.4% (2/27 larvae). Mean pupal development for *C. carnae* from the control and treatment groups was 12.17 ± 0.11 and 12.62 ± 0.10 days, respectively (P = 0.002). There were no significant differences in larval development, adult eclosion, sex ratio or larval mortality between individuals of the control and treatment groups. Results show that the microsporidium in *H. convergens* may be transmitted to lacewings under controlled conditions and this may have implications when both are used simultaneously for biological pest control. During pre-trial screening of *C. carnea*, larval mortality and adult deformities raised suspicions of the presence of a pathogen. A previously undescribed microsporidium was detected in prepared smears of symptomatic individuals. Molecular characterization of the pathogen revealed the microsporidium to be 96% similar to *Nosema granulosis*, a feminizing microsporidium described from the crustacean *Gammarus duebeni* Liljeborg. This unexpected discovery emphasizes the importance of ensuring that test individuals are free of pathogens before they are used in research trials.

Keywords: Microsporidia, lacewings, lady beetles

Poster / Microsporidia Monday, 14:00 M-10

Fire ant microsporidia acquired by parasitoid flies of fire ants

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The microsporidium *Kneallhazia* (formerly *Thelohania*) *solenopsae* and parasitoid flies in the genus *Pseudacteon* are natural enemies of the invasive fire ant, *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. Fire ant decapitating flies that developed in fire ants infected with *K. solenopsae* also acquired the pathogen. *K. solenopsae* was found in 51% of pooled samples, which included three species of flies: *Pseudacteon obtusus*, *Pseudacteon cultellatus*, and *Pseudacteon curvatus*. Field collected *P. curvatus* screened individually for infection had a prevalence of 12% which was not correlated to infection prevalence in fire ant populations. Attempts to demonstrate that *K. solenopsae* is vectored by the flies has thus far resulted in no transmission. Not all flies reared from infected ants acquire the microsporidium. In a sampling of *P. curvatus* at adult eclosion from infected ants, 24% (n=50) had *K. solenopsae*. *K. solenopsae* spores have been observed in adult flies reared from infected ants, however vegetative stages have yet to be found in fly tissue.

Keywords: *Solenopsis invicta*, red imported fire ant, phorid fly, Pseudacteon biological control, parasite, entomopathogen, microsporidia

NEMATODES

Poster / Nematodes Monday, 14:00 N-01-STU

Molecular cloning, expression and characterization of a serpine released by the entomopathogenic nematode *Steinernema carpocapsae*

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Steinernema carpocapsae is an entomopathogenic nematode that is able to invade, to overcome self-defenses and to induce insect death in about 48 hrs pos-contamination. The virulence of this pathogen is due to a large set of virulence factors expressed during the parasitic process. Serine proteases inhibitors were predicted by the analysis of expressed sequence tags (EST) and detected in the secreted / excreted products (ESP) of parasitic stage, thus it has been hypothesized to be a virulence factor. Based in EST sequences and 5' and 3' RACE a full cDNA of 1191 bp encoding for a serine-protease inhibitor was sequenced (*sc-serp-1*), which displayed high homology with *C. elegans* srp-7 and the *Trichinella spiralis* serpins. Expression analysis across nematode parasitic process showed that *sc-serp-1* was up-regulated in the initial phase of parasitism. This find combined with the fact Sc-SERP-1 was found in ESP supported the hypothesis it was participating in parasitism. To test for activity in insects recombinant protein was produced in *E. coli* Rosetta II (DE3). A recombinant protein with 40 kDa was produced, correctly refolded, purified using His-Tag and proved that the recombinant protein was codified by *sc-serp-1* by Maldi-MS/MS. Sc-SERP-1 inhibited partially trypsin and chymotrypsin activities. So far it was shown that Sc-SERP-1 caused slight

inhibition of propheneloxidase activity in insects, suggesting its participation in evasion.

Keywords: *Steinernema carpocapsae*, Cloning, Expression

Poster / Nematodes **Monday, 14:00 N-02**

Clotting factors and eicosanoids protect against nematode infections

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Entomopathogenic nematodes (EPN's) of the genera *Heterorhabditis* are obligate and lethal insect parasites. In recent years they have been used increasingly as biological control agents. Infective juveniles occur free living in the soil and are capable of seeking out hosts and penetrate them through the cuticle or natural orifices. EPN's are symbiotically associated with bacteria of the genera *Photorhabdus*. The bacterial symbionts are essential to kill the host (within 24-48 hours) and digest host tissues. *Drosophila* larvae are more resistant to nematode infection than *Galleria mellonella*, but both can be used as natural infection model.
The tripartite model (*Drosophila*, nematodes, bacteria) was recently established and used to show an immune function for transglutaminase, a conserved clotting factor. In this study we used different *Drosophila* mutants or RNAi lines with defects in clotting or other branches of the immune system. We demonstrated an immune function during nematode infection for known clotting substrates GP150 and Fondue. Our experiments show that compared to control animals *Imd* and *Bc* mutant larvae have similar viability after infection. In contrast double mutants in *Imd* and *Bc* show significantly higher mortality suggesting that phenoloxidase cooperates with the *Imd* pathway during the response to the nematobacterial complex. Furthermore, injection of eicosanoid biosynthesis inhibitors increases susceptibility to nematodes implying the importance of eicosanoids. In conclusion, we show that the *Heterorhabditis/Photorhabdus* infection model is suitable to identify novel regulators of innate immunity.
Our research is supported by grants from the Carl-Tryggers Foundation (U.T.) and Grant Agency of Czech Republic (GA206/09/P470).

Keywords: Drosophila, Heterorhabditis, immunity, clotting, eicosanoids

Poster / Nematodes **Monday, 14:00 N-03**

Innate immune responses of *Leptinotarsa decemlineata* and *Galleria mellonella* to the entomopathogenic nematodes, *Steinernema feltiae* and *Heterorhabditis bacteriophora* collected in Iran

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Hemocyte encapsulation reactions of infective juveniles of two Iranian isolates of the entomopathogenic nematodes (EPNs), *Heterorhabditis bacteriophora* and *Steinernema feltiae*, were compared in the economic pest, Colorado potato beetle, *Leptinotarsa decemlineata*, a resistant host and the greater wax moth, *Galleria mellonella*, a susceptible host. Complete encapsulation occurred by 4 h postinjection for *H. bacteriophora* in both *L. decemlineata* and *G. mellonella* and by 2 h pi for *S. feltiae* in *L. decemlineata*. The percentage of encapsulation from 4 h to 24 h pi in *L. decemlineata* was 84% for *S. feltiae* and 33% for *H. bacteriophora.* In *G. mellonella* there were no encapsulation or melanization responses against *S. feltiae*, whereas *H. bacteriophora* was encapsulated and melanized (17%), the encapsulation level being lower than in *L. decemlineata*. Such a study may contribute to effectively selecting EPN species active against significant economic pests based on the latter's cellular immune response.

Keywords: Cellular encapsulation, *Galleria mellonella*, *Heterorhabditis bacteriophora*, insect, *Leptinotarsa decemlineata*, melanization, nematode, *Steinernema feltiae*

Poster / Nematodes **Monday, 14:00 N-04**

Symbiotic bacteria can modify the competitive success of sympatric *Heterorhabditis megidis* and *H. downesi* populations

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Two closely related Heterorhabditis species, *H. megidis* and *H. downesi* can be isolated from same soil samples collected in forest habitats of Hungary. Both of these nematode species can be symbiotically associated with *Photorhabdus temperata* subsp. *temperata* and *P. temperata* subsp. *cinerea*, and in several places all the possible nematode-bacteria combinations can be found. Four EPN isolates (*H. megidis* with *P. t. temperata* bacteria, *H. megidis*/*P. t. cinerea*, *H. downesi*/*P. t. temperata* and *H. downesi*/*P. t. cinerea*) originated from the same location were used in a lab trial to study the competitive relations between the two nematode species. Different mixtures were made from the EPN isolates: 1000-0, 900-100, 500-500, 100-900 and 0-1000 IJs/ml of two nematode isolates in all possible combinations. In Petri dishes, on wet filter paper, 10 last instar larvae of *Galleria mellonella* or *Tenebrio molitor* were infected by the different nematode mixtures, in 5 replications, at room temperature. Mortality of test insects, reproductive success of nematodes (number of Ijs/insect larvae) and species identity of emerging nematodes were determined. The pathogenic and reproductive success of both nematode species were higher with *P. t. temperata* symbiont using *Galleria* larvae, while these parameters were higher with *P. t. cinerea* in the case of *Tenebrio* larvae. The competitve success of *H.*

megidis against *H. downesi* was higher independently of the symbiotic bacteria in the case of *Galleria* larvae, while the competitive success of *H. downesi* was higher with *P. t. cinerea* bacteria using *Tenebrio* test insect.

Keywords: *Heterorhabditis megidis*, *Heterorhabditis downesi*, competitive success

Poster / Nematodes Monday, 14:00 N-05

A survey study on entomopathogenic nematods in East Black Sea Region of Turkey

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Entomopathogenic nematodes (EPNs) are extensively used microbial control agents against the nocuous insects in all over the world. Utilization of the local isolates for controlling the pests found within the same geographical area has several advantageous including transportation and adaptation. In the current study, we have conducted a survey to isolate EPNs from a distinct region, East Black Sea Region of Turkey, where mainly covered by hazelnut plantation areas and forest. Out of 96 soil samples collected from the region, eight were positive for EPNs (8,3%). According to the morphological studies, it was determined that all of the samples were in Steinernema genus. The pure cultures of all nematodes were further identified based on ITS region sequence analysis. The samples were identified as *Steinernema carpocapsae* (1), *Steinernema feltiae* (5), *Steinernema* sp. (1) and *Steinernema kraussei* (1). Among these isolates, *S. feltiae* is the most frequently distributed species in the region. *S. kraussei* is recorded for the first time from Turkey. Our preliminary results also showed that *Steinernema* sp. may be a novel species, however, it could not complately identified yet. Further research will be directed to determine the microbial control potential of these isolates against the soil-borne insect pests in this region.

Keywords: Entomopathogenic nematode, Steinernema, ITS, Black Sea Region, Turkey

Poster / Nematodes Monday, 14:00 N-06

Effect of Essential oils on Entomopathogenic Nematodes

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Effect of thirteen essential oils (anise oil, clove oil, marigold, mustard oil, neem oil, quassia, quilaja, rosemary oil, rotenone, tea tree extract, thyme oil, wintergreen oil, and yucca) on entomopathobenic nematodes [*Steinernema carpocapsae* Pocheon strain (ScP) and *Heterorhabditis* sp. Gyeongsan strain (HG)] were investigated in the laboratory. Mustard oil was highly toxic to ScP and HG (100% mortality) at the concentration of 1,000 ppm in X-plate. The mortality of ScP and HG was below 20% by others essential oils at the concentration of 1,000 ppm two days after treatment in X-plate. 20 ppm of mustard oil resulted in 69.0% and 100% mortality of ScP and HG 3 days after treatment, but 4% and 36% at 5 ppm in X-plate, respectively. >200 ppm of mustard oil had effect on nematode survival and pathogenicity of ScP in sand column. On the contrary, >100 ppm of mustard oil had effect on pathogenicity of HG. Mortality of baited *Galleria mellonella* larva was not different from control at the concentration of 100 ppm of mustard oil while 30% lower in HG in sand column. Mean numbers of established infective juveniles of Hg in *Galleria* larva were lower than Sc in sand barrier.

Keywords: Essential oil, environment friendly agricultural materials, entomopathogenic nematode

Poster / Nematodes Monday, 14:00 N-07

Parasitism of *Eurygaster integriceps* Puton (Heteroptera: Scutelleridae) by *Hexamermis* sp. (Nematoda: Mermithidae)

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Sunn pest (SP), Eurygaster integriceps Puton (Heteroptera: Scutelleridae), is an insect of great importance on wheat and other Graminae in Turkey. Only a part of SP life cycle is spent in wheat fields. The adults of new generation move to mountains before harvesting wheat and barley. They have a rest under bushes and litter on mountains and become inactive in aestivation and diapauses about nine months. During this period various natural enemies and entomopatogenic diseases could play an important role in reducing populations of SP. Among them the mermithid parasite, Hexamermis sp. (Nematoda: Mermithidae) is the most important natural enemy in overwintering areas. The aim of this study was to determine the infection rate of SP with the parasitic nematode in 2008 and 2009. The adults of E. integriceps were collected from overwintering areas in the soil (about 0 - 5 cm) and under plant leaf litter in Gaziantep, Turkey. They were brought to laboratory in the ice box and sexed. Than they were dissected in distilled water to check for the presence or absence of mermithids the rates of parasitism were calculated individually females and males of the SP. The parasitism rates are 13.8% and 16.0% for females and 7.5% and 7.1% for males in 2008, 2009, respectively. Parasitized SP contained an average of 4.7.±0.79 (n=26) nematodes. In a sample of 26 parasitized SP 15.4% contained single worm infections, 34.6% contained 3 worms and 30.8% contained 5 or more worms. The mean body length of 23 juvenile samples that obtained SP were measured as 9.0 ± 0.65 cm (n=23). Hexamermis sp. is an important natural mortality factor of SP in overwintering areas of Gaziantep province. Our results suggest that Hexamermis sp. has potential as the biological control agent in SP management.

Keywords: Hexamermis, Eurygaster, Mermithidae, parasitic nematodes, sunn pest

Poster / Nematodes Monday, 14:00 N-08

Laboratory screening of the pathogenicity of some local entomopathogenic nematode isolates against the european cockchafer, *Melolontha melolontha* (Coleoptera: Scarabaeidae)

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The European cockchafer, *Melolontha melolontha* L. is one of the most important pests in agriculture and forestry in the Black Sea Region of Turkey, causing 15–20% damage annually in only hazelnut production. Increasing interest in developing environmentally safe pest control methods has inspired us to study the potential of entomopathogenic nematodes for controlling *M. melolontha*. When targeting the control of a specific insect pest within a region, it is very important to use the local isolate which is well-adapted to the ecological conditions as well as preventing the introduction of an alien biological control agent. In the current study, we screened the virulence of four local isolates of entomopathogenic nematodes (*Steinernema carpocapsae* B122, *S. feltiae* B1, *Heterorhabditis bacteriphora* M3 and *H. megidis* P69) against larvae of *M. melolontha* at three different temperature (15, 23 and 28 °C) at laboratory conditions. In bioassays using 6-well plates with sterile sand, insect larvae were exposed to a concentration of 1000 infective juveniles per individual. Maximum larval mortality (91.2%) was recorded from *H. bacteriophora* M3 at 23 °C, 75.7% from *S. feltiae* B1 at 15 °C and 64.6% from *H. bacteriophora* M3 at 28 °C. *S. carpocapsae* did not show any significant mortality against the larvae at any tested temperature. Future work will focus on the determination of the efficiency of the isolates against the pest in field conditions.

Keywords: *Melolontha melolontha*, Entomopathogenic Nematode, Biological Control, *Heterorhabditis bacteriophora*, Turkey

Poster / Nematodes Monday, 14:00 N-09

Control potential of *Heterorhabditis bacteriophora* against a new turf pest, *Dorcadion pseudopreissi* (Coleoptera: Cerambycidae) in turf

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Entomopathogenic nematodes (EPNs) offer an environmentally safe alternative control method. Nematodes in the families Heterorhabditidae and Steinernematidae are widely used as biological control agents of soil-inhabiting insect pests. In the study, the control potential and persistence of *Heterorhabditis bacteriophora* against a new pest *Dorcadion pseudopreissi* on turf was examined in field. Highest mortalities of the larvae were detected at the dose of 150 DL/larva in the laboratory. In the field experiment before nematode application, experimental turf parcels were checked if there were endemic EPNs in the areas. Afterwards the experimental parcels covered with cage (1 x 1 m) were involved with female and male *D. pseudopreissi* for ovipositon into the turf. After adult releasing into turf, *H. bacteriophora* was applied at 0.5 million DL/m^2 into turf species; *Lolium prenne* and *Festuca arundinacea* against this pest. The first results showed that differences of efficiency of *H. bacteriophora* between application areas and control for two kinds of turf was not significantly different ($p=0.330$ for *F. arundinacea* and $p=0.568$ for *L. prenne*), although lower insects were detected from applied areas. However, persistence of the nematode was observed for more than 5 months. Moreover, amount of damaged areas caused by the larvae on *F. arundinacea* were statistically significant between treated parcels with the nematode and control ($p=0.015$), whereas there was no significantly different on *L. prenne* ($p=0.658$).

Keywords: Biological control, *Dorcadion pseudopreissi*, *Heterorhabditis bacteriophora*, efficacy, turf, persistence, damage

Poster / Nematodes Monday, 14:00 N-10

Biological control potential of an entomopathogenic preperation of *Heterorhabditis bacteriophora* on the white grub, *Polyphylla adspersa*

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The white grub, *Polyphylla adspersa* (Col., Scarabaeidae) is one of the most important Scarabaeid pest of trees in North East of Iran. Long life cycle in three years and cryptic habitat made this pest as a major concern for different control methods. Entomopathogenic nematodes were considered as potential biocontrol agents against such this soil dwelling pest. In this study, a commercial formulation of Heterorhabditis bacteriophora (LARVANEM®) was examined on second and third larval instars and pupae of the white grub collected from urban parks of Mashhad, North East of Iran in 2009. The virulence of *H. bacteriophora* to larvae was assayed by applying 200 µl of distilled water containing 50, 200, 500 or 1000 IJs onto the soil surface of each cup. Control cups received same amount of water only. Larval mortality was assessed at weekly intervals for 4 weeks. Dead larvae were dissected under the stereomicroscope to confirm that the mortality resulted from nematode infection. The dead larvae were also kept on White traps to observe nematode emergence from nematode-killed insects. Second larvae were significantly more susceptible than third instar to H. bacteriophora. The mean mortality in larval stages was about 42%. Pupal stage of the white grub had high susceptibility to this entomopathogen.When we compared efficacy of other entomopathogenic nematode, *Steinernema feltiae* and *Steinerma*

carpocapsae, *H. bacteriophora* had the high efficiency. Larvae of this scarabaeid has evasive behavior with strong grooming. It is concluded that the pathogen, *H. bacteriophora*, has high potential for using in managing white grubs, *P. adspersa*.

Keywords: Susceptibility, Melolonthid, Entomopathogenic nematode, Iran

Poster / Nematodes Monday, 14:00 N-11

The effect of silver nanoparticles on the mortality of entomopathogenic nematodes from bioprepations

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Entomopathogenic nematodes are the natural component of soil mesofauna and an important factor limiting insect density. They are used in the production of biological preparations for controlling various pests. Steinernematidae and Heterorhabditidae are associated with mutualistic bacteria *Xenorhabdus* and *Photorhabdus*, respectively, which allow them to kill the host.
Nowodays development of nenotechnology is observed in the whole world. Nanotechnology is a discipline dealing with particles of 1 to 100 nm, which are named nanoparticles. Not much information is about the influence of nanomaterials on agrocenosis. The usage of nanomaterials makes possible the production of preparations with different biochemical properties. Nanotechnology has a great impact on biological sciences and more and more nanomaterials are used in medicine, pharmacy and agriculture. Silver is a noble metal which antibacterial properties have been known since the ancient times. In the ionic form, silver might be toxic for organisms but silver nanoparticles have a broad spectrum of biological properties even at low concentrations. The aim of these experiments was to study the effect of different concentrations of nano-Ag on mortality *of entomopathogenic nematodes Steinernema feltiae* and *Heterorhabditis bacteriophora* originated from biopreparations Entonem, Owinema and Larvanem, Nematop, respectively. The obtained results show high toxicity of nanoparticles on entomopathogenic nematodes (mortality: 98-100%), after the contact with concentration of 5 ppm. Lower concentration (2 ppm) also caused high mortality of infective juveniles (IJs) originated from biopreparation Owinema and Nematop - 99 and 100% respectively. While the lowest mortality (about 4%) was observed at the lowest concentration (0,5 ppm) in *H. bacteriophora* (Nematop).
Nanotechnology has opened new possibilities of the nanomaterials application in agriculture. This should boost better cognition of their influence on beneficial organisms and consequences of their application on natural environment.

Keywords: Entomopathogenic nematodes, EPNs, nano-Ag, nanoparticles

Poster / Nematodes Monday, 14:00 N-12

Sublethal impacts of Iranian isolates of *S. feltiae* and *H. bacteriophora* on the Colorado potato beetle, *Leptinotarsa decemlineata*

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Two isolates of entomopathogenic nematodes were recovered from 30 soil samples collected from suburbs of Tabriz, north-west of Iran and identified as *Steinernema feltiae* and *Heterorhabditis bacteriophora* based on morphological characters and morphometric data. To determine the LC_{50} values of the two entomopathogenic nematodes against *L. decemlineata* prepupae, different concentrations of the nematodes were tested against them in the soil. Due to the different temperature requirements of the two nematode species, bioassay experiments were conducted at 20±1 and 27±2 °C for *Steinernema feltiae* and *Heterorhabditis bacteriophora*, respectively. Both isolates were effective against *L. decemlineata*. LC_{50} values for progeny of over-wintering and laboratory generations of *H. bacteriophora* and laboratory generations of *S. feltiae* were estimated as 8.50, 7.65 and 51.24 IJ per prepupa, respectively. Cellular encapsulation of both nematode species was also observed. Sub-lethal nematode concentrations had adverse effects on CPB adult fitness manifesting as morphological deformation and delayed development.

Keywords: *Leptinotarsa decemlineata*, insect fitness, *Heterorhabditis bacteriophora*, LC50, *Steinernema feltiae*, sub-lethal concentrations

Poster / Nematodes Monday, 14:00 N-13

The new methods for study entomoparasitic nematodes in Georgia

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The new methods of entomoparasitic nematodes (EPNs) investigations have been developed at ARO, Israel (under the project CDR #TA-MOU-02-CA22-007 with supervision of Prof. I. Glazer) and the University of California Davis, USA (under the project CRDF/GRDF/GNSF #NSS-05-07 with guidance of Dr. E. Lewis). On the basis of new knowledge the experiments have been continued at the laboratory of Biocontrol, Kanchaveli L. Institute of Plant Protection, Georgia. The main goals of the proposed research was to develop of effective application techniques/tools, search the EPNs local strains and check the efficacy of nematodes against the main pest insects of urban horticultural plots, where the damage by pest insects has achieved the large scale. The sample materials of soil have collected from different zones of Georgia, such as: the vineyards region of Telavi, the fruit growing region of Gori and the urban plots of Tbilisi (East Georgia), citrus

orchards region and urban plots of Adjara and Guria (West Georgia). The soil samples have tested at laboratory conditions by using of sieving methods for nematode direct migration in water and baiting methods for hatching the infective juveniles (IJs) with last instars larvae of wax moth, *Galleria mellonella* and the meal worm, *Tenebrio molitor*. At present the local EPNs strains are detected by the sieving methods from the soil of Anaseuli citrus orchards (Guria region). The presence of nematodes was established. The invasive ability of isolated nematodes to the test larvae has been detected. The bioassays are continued to propagate the local strain for identification.

Keywords: EPN, Baiting, Wax moth - *Galleria mellonella*, Meal worm - *Tenebrio molitor*, Anaseuli

Poster / Nematodes Monday, 14:00 N-14

The susceptibility entomopatogenic nematode towards the mulberry moth

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The mulberry moth - *Glyphodes pyloalis* Walker ((*Lepidoptera: Pyralidae)* on the leaves of mulberry trees have been found in Kakheti (East Georgia). The pest is distributed in USA (Florida, Mississippi, and Virginia States), Mexico, India, Japan, Iran, in republic of Central Asia and Azerbaijan. The pest insect specialized as a monoplane damaging the mulberry plantings leaves. The *G. pyloalis* is considered as an urban pest and therefore it is recommended the application of environmentally safe means for mulberry trees protection. Among the entomopatogenic nematodes (EPNs) there is the very important species *Steinernema feltiae. S. feltiae* has proven particularly succesful and are now commercially mass-produced. The strain of *S. feltiae* was introduced in Georgia (Project CDR CA22-007, Israel), and then EPN has been mass produced successfully at Kanchaveli L. Institute of Plant Protection. The preliminary laboratory experiments on the susceptibility of *G. pyloalis* with S. *feltiae* have been carried out. The 200 individuals of IV instars larvae were collected from mulberry trees in Tbilsi (Park "Mziuri") and transfered at biocontrol laboratory in conditions 23-25°C and 70-75% RH. The nematodes suspensions 1000IJs/ml was used for treatment of mulberry leaves. The control being treated with sterile water. The mortality of *G. pyloalis* was corrected for control mortality using Abbott's formula. The invasive larvae were detected after 48 hr and mortality was achieved 64%. As the results of researches the susceptibility of EPN – *S. feltiae* to *G. pyloalis has* been established, which give possibility to use nematode suspension for control *G. pyloalis in* urban plots.

Keywords: Mulberry moth - *Glyphodes pyloalis*, *Steinernema feltiae*

Poster / Nematodes Monday, 14:00 N-15

Effect of copper on the flour beetle *Tribolium castaneum* resistance to the entomopathogenic nematode *Steinernema feltiae*

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The beetles were kept for one generation either in control medium or contaminated with 1000 mg Cu kg^{-1}. Next generation resistance to the nematodes was studied in two experiments.
In the first experiment differences between stages (larvae, females and males of pupae and adults) was examined. Studied parameters were: mortality and in the case of adults, respiration rate after infection. No effect of copper was observed but infection decreased all stages survival and the most sensitive were larvae (almost 100% mortality). At the same time, nematodes caused significantly higher pupae mortality in comparison to adults with any differences between sexes. Respiration rate of adults was the lowest in subjected to infection individuals from copper treatment with no interaction of both factors and any differences between sexes.
In the second experiment an influence of temperature (25ºC and 30ºC) on population parameters of infected/uninfected adults beetles were studied. Mortality was the highest in the populations from copper treatment subjected to infection and kept in 25ºC temperature. Reproduction was the lowest one week after infection, again in 25ºC in copper+nematode treatment. After two weeks from exposure to nematodes reproduction increased also in infected populations, being still the lowest in 25ºC in copper+nematode treatment.
The outcomes of presented study indicate that copper decreased sensitivity of beetles to the nematode infections.
The studies were performed under the EEA grants FRISC and project of Polish Ministry of Science and Higher Education Nr N N304 027334.

Keywords: Interaction pathogen contamination

Poster / Nematodes Monday, 14:00 N-16

The efficacy of *Steinernema feltiae* and *Steinernema carpocapsae* in controlling Colorado Potato Beetle on potato under field conditions

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We tested the efficacy of entomopathogenic nematodes in controling colorado potato beetle (*Leptinotarsa decemlineata*) on potato in a field experiment. We used Slovenian strains of nematode *Steinernema feltiae* (B30) and *Steinernema carpocapsae* (C101), azadirachtin (NeemAzal), commercial product based on toxin of Bacillus thuringiensis var. tenebrionis (Novodor) and thiametoxam (Actara). The application of the nematodes (100 000 Ijs/m^2) was repeated twice, while other substances were applied according to the orders

of the producers. Observing the population dynamics of Colorado potato beetle (0, 3, 7 days after the first application) we conclude, that entomopathogenic nematodes significantly decreased the number of larvae of the pest, while no effect on their eggs and adults was confirmed. When controling larvae insecticide thiametoxam showed the best results (0.45 larvae/plant), poor results gave control (12.55 larvae/plant) and azadirachtin (12.80 larvae/plant), meanwhile treatments with EPNs and Novodor showed no significant differences (*S. feltiae*: 7.30 larvae/plant, *S. carpocapsae* 5.45 larvae/plant, Novodor: 8.85 larvae/plant).

Keywords: *Steinernema feltiae*, *Steinernema carpocapsae*, *Leptinotarsa decemlineata*, biological control

Poster / Nematodes **Monday, 14:00**
N-17STU

Interactions between entomopathogenic nematodes (Steinernematidae, Heterorhabditidae) and the citrus nematode *Tylenchulus semipenetrans* (Tylenchulidae) in Arizona

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At present, 90% of citrus grown in Arizona are infected by the citrus nematode, *Tylenchulus semipenetrans*. However, given the current lack of successful control measures and the increasing awareness of the detrimental health and environmental effects due to chemical pesticides, new alternatives are deeply needed for management of this nematode. In this respect, one of the choices for substitution of chemical nematicides is the consideration of biological control agents such as entomopathogenic nematodes (EPN). In this study, two commercially available EPN, *Steinernema riobrave* (Biovector) and *Heterorhabditis bacteriophora* (Nemasys) were assessed for their efficacy against the citrus nematode in laboratory assays. Two-month-old rough lemon seedlings were grown in cone-tainers, at 25°C and 30% humidity. *Tylenchulus semipenetrans*-infected seedlings were inoculated at a concentration of 12,000 J2/seedling. Citrus nematode (J2) root penetration rate and female egg production were assessed in relation to: 1) EPN application time (i.e., simultaneous [EPN simultaneously applied with citrus nematode], after [EPN applied after citrus nematode establishment in the citrus seedlings]), and 2) EPN application method (i.e., aqueous suspension, EPN-infected cadavers). A completely randomized design with two replications (12 citrus-seedlings/treatment) for each evaluated parameter was considered. Data were subjected to analysis of variance (ANOVA). Results from these studies will be presented and discussed. Assessment of this information is critical for understanding EPN-citrus nematode interactions and in making predictions of the various impacts of EPN application for the control of this nematode.

Keywords: Citrus nematode, biological control, entomopathogenic nematodes

Poster / Nematodes **Monday, 14:00 N-18**

New insights in the pathogenic process of *Steinernema carpocapsae*: The role of proteases

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Steinernema carpocapsae is a pathogen that is virulent against a large number of insects. Transcriptomic analysis based in expressed sequence tags and secretome highlighted a large number of proteases predicted to participate in the parasitic process, thus constituting virulence factors. By in situ hibridazation and by quantitative –RT-PCR a set of these genes was proved to be expressed exclusively by the nematode parasitic stage. The mode of action of 5 of these proteases was achieved after native protein purification and production of recombinant proteins: two induced insect cell death, causing host tissue disarrangements and enabling nematode invasion; a third also contributed to invasion by promoting destruction of host's midgut basal lamina; the other two were shown to modulate evasion from insect innate-defences. Important to note that a large parte of the identified proteases presented a high number of postranscriptional modifications and also many of these proteases have more than one functional domain. We hypothesise that these multiple forms are playing distinct roles in distinct insects, thus supporting the ability the nematode has to parasitize a large spectrum of hosts.

Keywords: Proteases, *Steinernema carpocapsae*, virulence factors, mode of action

Tuesday July 13, 2010
Osman Turan Congress Center

06:00 **Bus pick up at hotels for 5K Runners**

06:30 **5K Fun Run/ Walk**

07:30 **Bus pick up at hotels**

Symposium Viruses
Tuesday, 8:00-10:00
Hasan Turan

Application of Insect Viruses in Medicine

Organizers: Linda King and Monique van Oers

Symposium Tuesday, 08:00 45

Opportunities and challenges in medical application of baculoviruses

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The baculovirus-insect cell expression system has traditionally been used to produce recombinant proteins, increasingly with the aim of producing subunit vaccines that may be monomeric surface proteins of viruses or self-aggregating virus-like particles (VLPs). An example is a human cervical cancer vaccine based on the L1 protein VLPs of human papillomavirus. Displaying foreign proteins on the surface of baculovirus particles is another way to make potent vaccines. Vaccines ideally are accompanied by diagnostic kits that allow discrimination between infected and vaccinated individuals. The antigens for these and other diagnostics are often made with baculovirus vectors too. Although baculoviruses do not replicate in mammalian cells they can enter mammalian cells and, hence, they can be used as gene delivery vectors. As such they have direct applications for gene therapy, delivery of intracellularly expressed antigens and delivery of lentivirus vectors. A new area of gene delivery being investigated is in organ transplantation. The advantages of using baculoviruses for mammalian gene transfer is that they can incorporate large amounts of foreign DNA, pre-existing immunity is absent and these viruses penetrate deep into tissues or organs. Baculoviruses also appear to have a preference for tumor cells, making them suitable for treatment of cancer. Baculovirus surface modifications may assist in targeting specific cells or prevent inactivation by the complement system. Newly developed methods for modifying the baculovirus genome at various locations allow the tailoring of the virus for each specific purpose.

Keywords: Baculovirus, vaccine, gene therapy, mammalian cells

Symposium Tuesday, 08:30 46

A fast track influenza vaccine made in insect cells

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Protein Sciences is developing FluBlok, a recombinant HA vaccine produced in cell culture using the baculovirus vectors system. FluBlok provides an attractive alternative to the current egg-based influenza vaccine (TIV) manufacturing process and presents the possibility for safe and expeditious vaccine production.
The high purity of the antigen enables administration at higher doses without a significant increase in side-effects in human subjects. The HA genes from the annual WHO recommended strains are cloned, expressed and purified using a general purification process.
The insect cell - baculovirus production technology is a modern solution for rapid antigen production and this technology is particularly suitable for influenza where annual adjustment of the vaccine is required or to address health care emergencies as currently posed by the H1N1 swine flu influenza virus.
The speaker will discuss the development of FluBlok, a trivalent recombinant hemagglutinin influenza vaccine. In addition she will address Protein Sciences' efforts to develop a rHA vaccine against the H1N1 A/California /04/2009 in record time and the Companies plans to transfer technology to other countries/locations to ensure "true" pandemic preparedness.

Keywords: Influenza, baculovirus, influenza virus, insect cells

Symposium Tuesday, 09:00 47

Humanised Baculoviruses for Cancer Gene Therapy

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Transfer of cytotoxic genes into human cancer cells offers an alternative form of treatment to complement surgery, radiotherapy and chemotherapy. Normally, viruses native to humans, which have adapted to infect and grow in the human host are employed as vectors. However the most commonly used vectors: type c adenoviruses, are expensive to generate, have a limited exogenous DNA capacity, and are required to infect patients who already have an immune memory, since most of the human population have already been exposed to common adenovirus infection.
As part of the BACULOGENES FP6 research project, we have generated baculoviruses (Bv) with a human tropism. By incorporation of linker molecules and/or growth factor receptor ligands into the budded virus envelope protein gp64, an enhanced attachment specificity for specific human cell types can be achieved, although unmodified Bv display a remarkable preferential affinity for human cancer cells. Insertion of intact human transcriptional control

sequences to drive therapeutic genes is also facilitated by the capacity of Bv vectors to accept large fragments of exogenous DNA. No Bv replication and minimal expression of native Bv genes are detected. The humanized Bv vectors show a remarkable ability to penetrate into human tissues and tumours, in a situation where adenoviral vectors follow either an injection track or infect only superficial epithelial layers, thus failing to infect either tissue or cancer stem cells. For *in vivo* use, the potent complement response against Bv in mammals has been eliminated by incorporation of a combination of genetic and chemical inhibitors.

Keywords: Baculovirus, Gene Therapy, Cancer

Symposium Tuesday, 09:30 48

Novel approaches in producing adeno-associated virus in insect cells

Cristelle Rivière[1]; **Nicolas Laroudie**[1]; **Martin Marek**[1]; **Loic Millot**[1]; **Lionel Galibert**[1]; **Mehdi Gasmi**[1]; **Otto-Wilhelm Merten**[1]
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Adeno-associated virus (AAV) belongs to the family of the *Parvoviridae* and is one of the most interesting viral vectors for gene therapy purposes of inherited and acquired diseases. Recently some very exciting clinical outcomes, for instance for the treatment of rare ocular diseases such as Leber's congenital amaurosis, have been communicated. The amount of AAV vector necessary for localized applications can easily be produced by traditional transfection methods using mammalian 293 cells. However, these traditional methods are insufficient to produce the number of AAV particles required for the treatment of neuro-muscular diseases, such as Duchene's muscular dystrophy (estimated dose: $1\text{-}5x10^{13}$ vector genome/kg). The Sf9 cell/baculovirus system is the most promising production system for large scale production of AAV vectors. This AAV production system was developed in the early 2000s (R. Kotin, NIH) and is based on three baculovirus vectors together encoding the replicase and capside functions of AAV as well as the recombinant AAV-vector. The Sf9 cells growing in suspension are infected at a density of 10^6 cells/ml. Three days post-infection, the AAV vector is harvested and purified. This production system is easily scalable and 50L scale runs using disposable stirred tank reactors are routinely performed. Such production runs provide total AAV1 vector amounts up to $2x10^{15}$ vg with concentrations reaching $5x10^{13}$ vg/ml. Different ways (e.g. the recently developed 2 baculovirus system or the use of a high cell density culture process) for optimizing this vector production system with respect to increasing vector quantity per run together with the highest quality in view of clinical applications will be presented.

Keywords: Adeno-associated virus (AAV), clinical outcomes

Symposium Nematodes Tuesday, 8:00-10:00
Nihat Turan1

Biotic and Abiotic Determinants of Entomopathogenic Fungi: Where Have All the Nematodes Gone?

Organizers: Ed Lewis and Selcuk Hazir

Symposium Tuesday, 08:00 49

Persistence on a large scale: soil type, predators and alternate hosts

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Entomopathogenic nematodes (EPNs) are insect parasites used in biological control. Many aspects of their biology are well-understood, especially under laboratory conditions, but what happens to EPNs after they are applied to crops remains poorly understood. For example, sometimes applications of EPNs persist for years in the same place by recycling through hosts, while at other times, they seem to disappear after just a few days. A long-term objective of our research group is to uncover the reasons for this disparity in field persistence that has been so well documented. Here, I will discuss field and laboratory studies exploring two potential regulators of EPN persistence: (1) physical and chemical aspects of soil and (2) soil food webs containing potential hosts and predators of EPNs. EPNs' ability to infect hosts varied among soil types. We also found significant correlations between soil characteristics (texture and physical characteristics) and EPN foraging efficacy. We can predict EPN success in various soil types. Measuring biotic regulators of EPN persistence showed that extant non-target arthropods may contribute to variation. Hosts of EPNs may increase persistence and/or predators of nematodes may decrease persistence. We found significantly fewer earwigs under treated trees where nematodes were applied one week previously, suggesting that earwigs are an alternate host. We found significantly more isotomid collembola and predatory mites under treated trees. Collembola and mites may opportunistically eat the nematodes, decreasing their effectiveness as biological control agents. How these arthropods interact with edaphic factors may hold the key to explaining EPN persistence.

Keywords: Entomopathogenic Nematodes, persistence, efficacy, soil

Symposium Tuesday, 08:30 50

Efficacy and persistence of entomopathogenic nematodes for controlling white grubs in peanut fields in Israel

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White grubs of *Maladera matrida* Argaman (Coleoptera: Scarabaeidae) are major soil pests of agriculture crops causing substantial damage to ornamentals, peanuts and sweet potatoes. Two trials were conducted in peanut field during the growing season of 2009 (April to October) in the north-western "Negev" region of Israel. We evaluate the efficacy and persistence of commercial entomopathogenic nematodes products by Koppert Co., Holland. The nematodes were applied using a back sprayer once, twice or three times during each flying period of the adult pests. The presence of nematodes in the soil was evaluated twice using 'Galleria traps'. The affect of the various treatments on yield and damage to the peanuts were determined at harvest time. Application of 'ENTONEM', containing *Steinernema feltiae,* did not affect the yield and damage, whereas application of 'LARVANEM', containing *Heterorhabditis bacteriophora,* resulted in 80% reduction in damage to the peanuts with no affect on the yield. In both trials, nematodes appeared to be active during the entire growing season. Towards the end of the season, nematode activity spilled over to the un-treated control plots.

Keywords: White grubs, peanuts, efficay, EPNs

Symposium Tuesday, 09:00 51

Potential negative effects of *Sancassania polyphyllae* (Acari: Acaridae) on entomopathogenic nematodes

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Sancassania polyphyllae (Acari: Acaridae) uses the larva of the white grub, *Polyphylla fullo* (Coleoptera: Scarabaeidae), as a phoretic host for its dispersal. It occurs as a non-feeding, deutonymphal phoretic stage or *hypopus* and continues its life cycle upon death of its phoretic host. The mite feeds as a scavenger on the host tissues but is also known to feed on the infective juveniles (IJs) of entomopathogenic nematodes in the families Steinernematidae and Heterorhabditidae. These nematodes are important biological control agents of soil insect pests. The objectives of our study were to (1) evaluate the predation by mites on *Steinernema feltiae* IJs (Rhabditida: Steinernematidae) from nematode-killed insects on agar and in soil column and (2) assess predation efficiency of the mites on IJs alone in a soil column. We documented that 96% of the emerged IJs from an insect cadaver were consumed by 10 adult female mites. In a soil column with a nematode-killed insect, the average number of IJs recovered was <30 when mites were present; however, the average number of IJs recovered was >375 when the mites were absent. When the IJs alone were placed in the soil column with mites for 4 and 10 days, their recovery from soil differed depending on where the IJs were placed in relation to the mites. *S. polyphyllae* was not as efficient at finding the IJs when they were separated from each other in the soil. Our data suggest that the mites were cueing in on the cadaver as a food source resulting in consumption of the IJs.

Keywords: Insect-parasitic nematode, nematophagous mite, *Polyphylla fullo*, *Steinernema feltiae*, white grub

Symposium Tuesday, 09:30 52

Do arthropod scavengers consume nematode-killed insects?

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Entomopathogenic nematodes of the families Steinernematidae and Heterorhabditidae are effective biological control agents for the control of soil-inhabiting insect pests. After the infective juveniles along with the mutualistic bacteria infect and kill an insect host, the cadaver remains in or on the soil surface for 1 or 2 weeks before the new generation of infective juveniles emerges from the host. During this period, we hypothesized that the cadavers are at risk of being consumed by arthropod scavengers. Therefore, we evaluated the following arthropods including a species of ant, cockroach, cricket, mite, and wasp, as potential scavengers of nematode-killed insects. Larvae of the wax moth, *Galleria mellonella*, infected by *Steinernema feltiae* or *Heterorhabditis bacteriophora* along with its respective mutualistic bacterium (i.e., monoxenic nematodes) were used in the experiments. Depending on the experiment, nematode-killed larvae that were dead for 1 to 9 days were exposed to the potential scavengers in the laboratory or field. Also depending on the experiment, larvae killed by freezing or by *S. feltiae* or *H. bacteriophora* without its respective mutualistic bacterium (i.e., axenic nematodes) served as controls. The species of ant, cockroach, cricket, and wasp consumed only dead control larvae and one-day-old nematode-killed larvae, whereas the species of mite consumed all cadavers. We conclude that the insect scavengers are deterred from feeding on nematode-killed larvae a (i.e., with the mutualistic bacteria) greater than 2 days old but will feed on frozen-killed larvae and larvae killed by axenic nematodes. Moreover, our data suggest that the deterrent to the insect scavengers is associated with the mutualistic bacteria.

Keywords: Scavengers, entomopathogenic nematodes, biological control, natural enemies

Contributed papers

Tuesday, 8:00-10:00
Fahri Kuran

FUNGI 1

Chairs: Richard Humber and Ali Sevim

Contributed Paper Tuesday, 08:00 53

Study on the characteristics and pathologenicity of the entomopathogenic fungus *Beauveria bassiana* as biological control agent of *Bemisia tabaci*

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Bemisia tabaci (Gennadius) is ranked among the most noxious pests attacking field and greenhouse crops around the world. The entomopathogenic fungus *Beauveria bassiana* is a main agent of the biological control of *B. tabaci*, five isolates of *B. bassiana* were evaluated for their characteristics and pathogencity to find out the most effective one. Mycelia growth rate and sporulation yield of the five isolates were significantly different from each other after 20 days on artificial media SDAY under lab conditions, and the isolate Bb62 has the strongest potential in mycelia growth rate and sporulation yield followed by the isolates Bb325, Bb324, Bbb2, and Bbb1 with values of mycelia growth rate 3.58, 3.11, 2.83, 1.87, and 1.46 mm/day, and sporulation values 20.25, 18.13, 16.25, 11.75, and 10.35×10^7 conidia/cm^2, respectively. With five concentrations 10^4, 10^5, 10^6, 10^7 and 10^8 conidia/ml of each isolate the mortality of the second-instar of *B. tabaci* differed significantly. Mortality caused by Bb62 isolate was the highest one among all of the isolates at 6 and 10 days after suspensions application. The pathogenicity varied also significantly, the LC_{50} values were 5.37×10^4, 1.51×10^5, 3.16×10^5, 3.71×10^6, and 5.88×10^6 conidia/ml respectively. The LT_{50} values were the shortest for Bb62 with 5.67 d.

Keywords: *Bemisia tabaci* (Gennadius), entomopathogenic fungus

Contributed Paper Tuesday, 08:15 54

Back to biology: fungal pathogens in mosquito management

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While we are challenged with escalating levels of mosquito resistance to conventional chemical pesticides, new emphasis on exploiting biologically-based adulticides for vectors of malaria and dengue is generating novel approaches to mosquito control. Many attributes of mosquito-specific fungi such as their relatively slow speed-of-kill, the multiplicity of toxins produced, and their low toxicity to non-targets makes them particularly advantageous. This presentation will outline different strategies used to control adult vectors using entomopathogenic fungi in different environments. When adult *Aedes aegypti* are killed by the ICPIPE 30 strain of *Metarhizium anisopliae*, no fungal spores are evident on the external cuticle of the mosquito. By 7 days after the mosquito dies, fungal spores have erupted on the integument for transmission to new hosts. Strategies for improving rates of fungal transmission and persistence in the environment are needed.

Keywords: Entomopathogenic fungi, Metarhizium, mosquito, *Aedes aegypti*, yellow fever mosquito

Contributed Paper Tuesday, 08:30 55

Research on biological efficiency of entomopathogenic fungi *Fusarium subglutinans* against *Aphis fabae* (Hemiptera: Aphididae)

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In this study, biological efficiency of entomopathogenic fungi *Fusarium subglutinans* isolated from cotton aphid was evaluated against *Aphis fabae* (Aphididae: Hemiptera) on bean in climate chamber. Two isolates (12, 21) of *F. subglutinas* were cultured in Potatoes dextrose liquid medium and were incubated in 4days. At the end of this period fungus suspension in Erlenmeyer was passed through cheesecloth and centrifuged for 5 minutes The pellets were diluted with Potatoes dextrose liquid medium containing Tween 20. Three different concentrations of each isolates ($1X10^6$, $1X10^7$, $1X10^8$ spore/ml) were prepared. Spore suspension of each isolates was applied with a compressor to aphids on bean plants. Potatoes dextrose liquid medium containing Tween 20 was applied to control plants After experimenting, plants were put in polyethylene bags moistened with water and incubated at 25 0C , 16 hours light / 8 h dark conditions in 24 hours. Live and dead aphids were recorded from leaf samples in 1st, 3rd, 5th, 7th, 9th, 11th, 13th, 15th days. Three different concentrations of two isolates (12, 21) of *F. subglutinas* were observed significant differences in aphid mortality at 25 ^{0}C. The highest mortality rate was calculated in $1X10^6$ spore / ml. There were no differences in mortality rate between $1X10^7$ and $1X10^8$ spore / ml concentration. The pathogenicty was carried out of Fs 21. In climate chamber experiment with these two isolates, aphid population was controlled 2 weeks later after application.

Keywords: Entomopathogenic Fungus, *Aphis fabae*, *Fusarium subglutinans*, biological control

Contributed Paper Tuesday, 08:45 56

Comparison of formulations of entomopathogenic fungi for treatment of artificial hideouts for biocontrol of *Cydia pomonella* and *Cydia funebrana*

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Cydia funebrana is a serious pest of plum fruits in organic agriculture. Therefore, we investigated whether this pest can be controlled by artificial hideouts treated with entomopathogenic fungi under lab conditions. Because we were not able to establish a mass rearing of *C. funebrana* we did additional experiments with the related species *C. pomonella*. We tested corrugated cardboards and different mulch substrates on acceptance for pupation. The results indicate that the moths accepted corrugated cardboard and especially bark mulch. We also did comparative experiments with oils and tensides. Both insect species were sensitive to the formulation. In case of *C.*

pomonella a mortality of 46% and 92% was determined for Tween80 and sunflower oil, respectively. The addition of conidia of *Beauveria bassiana* did not enhance the mortality but even when low concentrations of conidia were applied in oil, 90% mycosis was achieved. In contrast high concentrations of conidia were needed to achieve at least 70% mycosis when formulated in water containing Tween 80. First experiments were set up with the re-isolate of the commercial product Naturalis-L. Because we did not achieve high mortality rates with *B. bassiana* we tested other entomopathogenic fungi like *Lecanicillium lecanii*, *Paecillomycis fumosoroseus* and *Metarhizium anisopliae*. *M. anisopliae* and *P. fumosoroseus* showed the highest mortality rate.Furthermore, *C. pomonella* seems to be more sensitive to entomopathogenic fungi than *C. funebrana*. The presented data demonstrate that vegetable oils have a dramatic effect on larvae and oil-based formulations can improve the efficacy of entomopathogenic fungi.

Keywords: Entomopathogenic fungi, Cydia, formulation, hideouts

Contributed Paper Thuesday, 09:00 57

Entomopathogenic fungi as a potential biological agents for the control fall webworm – *Hyphantria cunea* Drury (Lepidoptera: Arctiidae)

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The fall webworm, *Hyphantria cunea* (Lepidoptera: Arctiidae) is one of the economical damaging pest insect in Georgia. In laboratory (2008), against 4th and 5th instars larvae of *H. cunea*, indigenous isolates of entomopathogenic fungi *Beauveria bassiana* (BbCPB-04) and *Metarhizium sp*. (MGS-1) were tested. Fungal Suspensions of the isolates were prepared from 2 week-old cultures grown on PDA, in 0.01% (w/v) Tween 80 and adjusted to $5x10^7$ conidia $^{ml-1}$. The larvae were immersed in conidial suspension, place in glass jar with tree leaves and kept at room temperature with 16/8 light / dark regime.
They were daily checked to detect symptomatic or dead larvae, which were removed and placed individually in Petri plates with moisture environment for develop of conidia. For the most virulent isolates infection rate from *B.bassiana* were 75% and from *Metarhizium sp* 82%.
The living larvae which hided under leaves and cordon made cocoons, where transform in pupae. They have been left overwinter until spring in the 8±5 º C condition.
Adult moth appeared from the pupae, after 4-5 day emerged massive and mate. The eggs were laid in masses which was continue 10-12 day. The emergences adults in variance of *B. Bassiana* was 69.6%, *Metarhizium sp*. 60%, in control - 55.7%. The larvae hatched seven - ten days later (*B.Bassiana* was 76.3%, *Metarhizium sp*. 70%, Control 89.5) and began feed fresh leaves intensively in group.
The results suggest that the *B.Bassiana* and *Metarhizium sp*. isolates may be particularly useful to control *H.cunea*.

Keywords: Keyworeds; *Hyphanthria cunea*, *Beauveria Bassiana*, *Metarhizium* sp.

Contributed Paper Tuesday, 09:15 58

Development of Met52 for the control of sucking insect pests in North America and Europe

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The entomopathogenic fungus *Metarhizium anisopliae* strain F52 is currently sold in the U.S. as Met52 and in Europe as Bio1020 for the control of black vine weevil in hardy nursery stock and berries. Products based on this isolate are currently under development in North America and Europe for a wide range of insect pests. Options for the effective control of thrips have become limited due to a reduction in available active ingredients from regulatory restrictions and insecticide resistance. Thrips are therefore an attractive target for the development of products with a new mode of action. An emulsifiable concentrate formulation based on *M. anisopliae* strain F52 is being developed for control of foliar and soil-dwelling life stages of thrips. The formulation has demonstrated consistent efficacy against thrips in a range of fruit, vegetable, and ornamental crops. Use patterns are currently being refined including application timing, placement, compatibility with beneficials, and rotation with insecticides. These new products offer an attractive new tool for the integrated pest management and resistance management of thrips and other sucking insect pests.

Keywords: *Metarhizium anisopliae*, thrips, Met52, Bio1020, whitefly, mite

Contributed Paper Tuesday, 09:30 59

The inhibitory effect of the fungal toxin, destruxin-A, on behavioural fever in the desert locust, Schistocerca gregaria

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The desert locust, *Schistocerca gregaria*, is a widespread agricultural pest. Variable success rates of a biopesticide based on the fungal pathogen *Metarhizium anisopliae* var *acridum* (IMI330189) have been attributed to the locusts' ability to thermoregulate. By selecting higher environmental temperatures during an infection, locusts can generate a behavioural fever. This can be both directly detrimental to the pathogen and enhance other aspects of the host immune response, thereby improving survival. Infrared technology was used to investigate the development of behavioural fever during mycosis with IMI330189. A behavioural fever was established from 22 hours post-infection and was sustained throughout daylight hours. However, only a short-lived fever was observed for locusts infected with the related fungus *M.a.* ARSEF 2575. Unlike IMI330189, the pathogenicity strategy of ARSEF 2575 has been largely attributed to the production of toxins, particularly destruxins. Co-administration of IMI330189 with destruxin-A, had an inhibitory effect on behavioural fever and reduced survival rates. This was particular evident when destruxin-A was administered early on in the infection process. Currently, the mode of action of destruxins is poorly

understood. The inhibitory effect on fever seen here may provide insights into mechanisms involved in pathogenicity.

Keywords: Behavioural fever, metarhizium, destruxin-A, desert locust

Contributed papers
Tuesday, 8:00-10:00
Nihat Turan 2

MICROBIAL CONTROL 1

Chairs: O. P. Perera and Fikrettin Sahin

Contributed Paper Tuesday, 08:00 60

Toxicity of the *Yersina entomophaga* super toxin complex (YeSTc) on midgut cells of *Costelytra zealandica* (Coleoptera: Scarabaeidae)

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A novel gram-negative, rod-shaped, non-spore-forming bacterium (*Yersinia entomophaga* MH96) (Enterobacteriaceae) was isolated from diseased larvae of the New Zealand grass grub, *Costelytra zealandica* (Coleoptera: Scarabaeidae). *Y. entomophaga* produces a proteinaceous toxin (YeSTc) which is responsible for mortality in a range of insect species, mainly within the Coleoptera and Lepidoptera. The YeSTc genes are members of the Toxin complex (Tc) toxin family, with orthologs identified from several other bacterial species including *Serratia entomophila* (Sep), *Photorhabdus luminescens* (Tca, Tcb, Tcc, Tcd), *Xenorhabdus nematophilus* (Xpt), and other *Yersina* spp. (e.g. *Y. pseudotuberculosis*, *Y. pestis*). While the mechanism of YeSTc activity remains unknown, histopathological examination of *C. zealandica* larvae after YeSTc treatment revealed a progressive deterioration of the midgut epithelium. This is in marked contrast to the lack of any effect observed by the *S. entomphila* Sep protein complex, which is the causative agent of the amber phenotype in amber disease. Among the distinct series of events observed, the midgut columnar cells lost their orderly rectangular form with the cells taking on an oval, slightly ragged appearance. This coincided with the apparent sloughing of cell-like vesicles into the gut lumen, which ultimately led to a complete breakdown of the midgut epithelium. We have examined the basis for the YeSTc toxicity using a number of cellular and physiological indicators (including 2D gel, enzyme activity, and intracellular processes) and will discuss our results in relation to what is known from other members of the Tc family.

Keywords: Scarab midgut, Toxin complex proteins, histology, proteomics, enzyme activity, *Yersinia entomophaga*, *Costelytra zealandica*

Contributed Paper Tuesday, 08:15 61

Three new strains of *Bacillus sphaericus* as potential biocontrol agent against mosquitoes

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Bacillus sphaericus, is known to be effective against *Culex* spp. and *Anopheles* spp. , and has better residual activity in polluted waters by production of binary toxin (Bin) and mosquitocidal toxins (Mtx). In this study, three new strains of *B.sphaericus* (MBI5, 6, 7) isolated from some greenhouse pests and larval habitat of Istanbul, Turkey in the spring and summer of 2008. In laboratory bioassays, all of the strains were able to kill larvae of *Culex* spp. and *Aedees* spp. in water (regardless of presence of organic matter), even 4 weeks after the application. The larvicidal activity of the strains were found to be related to the presence of four toxin genes, Bin A, Bin B, Mtx 1 and Mtx 2 in MBI 5, MBI 6 and MBI 7 strains. Moreover, the effectiveness of the three bacteria was tested against nematodes (*Meloidogyne arenaria*) that live on the roots of *Lycopersicon esculentum L.*. In nematode tests, plant extracts which have known to have lethal effects on the greenhouse pests, were also studied. Each *B.sphaericus* strain identified in this study were characterized as unique and novel in terms of BIOLOG, FAME profiles and 16S rDNA sequencing data. Toxicology tests were also performed on rats and the strains were determined as safe. The results of this study suggested that the three strains of *B. sphaericus* may be new sources of potential biocontrol agent of mosquitoes.

Keywords: *B. sphaericus*, mosquito, microbial control, nematode

Contributed Paper Tuesday, 08:30 62

Effect of *Bacillus thuringiensis* as vegetative form on hemocytes of *Rhynchophorous ferrugineus* (Coleoptera Curculionidae) larvae

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The bacterium *Bacillus thuringiensis* (*Bt*) is a pathogen of many insect species and is actively used in biocontrol. The vegetative form as been reported to be involved in insect septicemia process. *Bt* during the vegetative stage of growth, is known to secern a new family of insecticidal proteins. Moreover recently evidence has been provided, that *B. thuringiensis* can establish itself in replicative and vegetative form on the leaf surface. Little is known on the interaction of pathogens with the defense responses of phytophagous insects. Insect circulating hemocytes are primarily responsible for the immune defense against parasites and pathogens. We use as model *Rhynchophorous ferrugineus* Olivier (Coleoptera: Curculionidae) to study the interaction between Bt and insect hemocytes. *R. ferrugineus* is a key pest of palm trees who are still difficult to combat with both chemical and biological

control. Here, we report on the response of larvae of *R. ferrugineus* circulating hemocytes following Bt vegetative form inoculation. In the hemolymph, plasmatocytes, granulocytes, prohemocytes, oenocytes and spherulocytes were identified. After injection of a sub lethal dose, of Bt, RPW larvae had a decrement in the total number of circulating hemocytes. Particularly there was a decrement in the plasmatocytes which also lost their typical spindle-shape as shown by light and TEM microscopy. However further research are necessary to clarify the role of plasmatocytes in the larvae and their interaction with Bt.

Keywords: Red Palm Weevil, Bt, Hemocytes, Biocontrol

Contributed Paper Tuesday, 08:45 63

Identification, tissue expression patterns, and genomic structure of alkaline phosphatase genes in the Corn Earworm, *Helicoverpa zea*

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Membrane-bound forms of alkaline phosphatases (mALPs) have been identified as functional receptors for Cry toxins from the bacterium *Bacillus thuringiensis.* We have identified two forms of ALPs in *H. zea* and studied relative expression levels of ALPs in different tissues using quantitative real time PCR. Here we report cDNA sequences and tissue expression patterns of two ALP genes in *H. zea*. *C*omparative analysis of the genomic structure of ALP genes of *H. zea* and *H. virescens* is also presented.

Keywords: Helicoverpa, bollworm, alkaline phosphatase

Contributed Paper Tuesday, 09:00 64

Use of *Bacillus thuringiensis israelensis* for control of the European crane fly *Tipula paludosa* (Diptera: Nematocera)

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Tipula paludosa (Diptera: Nematocera) is a major pest in turf grass in temperate climates in North West Europe and North America. Results obtained during the COST Action 862 are summarized. Pathogenicity of insecticidal crystal proteins (ICPs) was assessed. Results of greenhouse and field trials will be presented. Quality control quantifying ICPs using monoclonal antibodies are compared with other methods.

Keywords: *Tipula paludosa*

Contributed Paper Tuesday, 09:15 65-STU

Characterization of insects intracellular response to *Bacillus thuringiensis* Cry toxins

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Pest insects can damage agricultural crops, consume and/or damage harvested food, and cause illness as vectors of human disease. The bacterium *Bacillus thuringiensis,* commonly used as biological control agent, produces proteinaceous Cry insecticidal pore forming toxins (PFT). Bravo´s model proposed that Cry toxins binding secuentially to different membrane receptors leads to cell dead by pore formation. Expanding on this model, novel work proposes that PFT (including Cry) act as trigger agents of cellular signaling in response to toxin injury. The p38 pathway has been identified as an important pathway in cellular stress response. In the present work we started the elucidation of insect intracellular response to Cry toxins, beginning with the study o MAPK/p38 role in insect defense after Cry challenge. We found that p38 transcription, translation and activation by phosphorilation in *M. sexta* and *Ae. aegypti* larvae midgut cells were induced by Cry. Gene silencing of p38 in both larvae resulted in hypersensitivity to specific Cry. Therefore, p38 plays a role in Cry toxin survival. Proteomics allowed the identification of proteins involved in response to Cry. Using a proteomic approach, DIGE, to analyse changes in protein expression associated with exposure to Cry11A CL_{10} and Cry11A CL_{50} in midgut mosquito cells were identified several differentially-expressed proteins. Our next goal is to determine the functions of these proteins in insect cell response to Cry. The RNAi techniques developed in this work will allow us to interfere the target proteins, and in consecuence will allow us to propose new elements about insect cell response to Cry and in the other hand, to design complementary methods to biocontrol. Because we showed that it is posible to enhance Cry toxin action by inhibiting a specific signal transduction pathway, this may have biotechnological applications, for example, enhancing the activity of some Bt Cry toxins against specific insect pests, by feeding larvae at the same time the specific MAPK p38 dsRNA and the Cry toxin by co-expressing both factors in the same transgenic plant.

Keywords: Cry, *Bt*, PFT, celullar response, *Ae. aegypti*, *M. sexta*, signal transduction, biocontrol

Contributed Paper Tuesday, 09:30 66

Long-term benefits of GM crops: Potential for *Diabrotica* suppression in Europe using Bt Maize

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Transgenic crops producing insecticidal proteins from *Bacillus thuringiensis* (Bt) have been widely adopted since 1996 in the United States of America to combat important pests of maize and cotton. There is growing evidence that several target pest populations have been dramatically reduced in areas where the Bt crops have been most intensively adopted over multiple years. The evidence is most dramatic for non-migratory monophagous and oligophagous species that show high mortality on Bt crops, such as European corn borer (*Ostrinia nubilalis*), and tobacco budworm (*Heliothis virescens*). Bt cotton is currently being used in the southwestern USA and Mexico as part of an area-wide eradication program for pink bollworm (*Pectinophora gossypiella*). SmartStax™ Bt maize line producing coleopteran-active insecticidal proteins have been shown to cause >99% mortality of western corn rootworm (*Diabrotica virgifera virgifera*) larvae every year. Simulation models suggest that long-term area-wide cultivation of these Bt corn lines can lead to dramatic population reduction, and even local extinction, of corn rootworms. Area-wide suppression of this economically important pest, based around Bt maize and incorporating a combination of other tools, would benefit European agriculture and the environment. Such long-term benefits to agricultural production systems can be reasonably expected from the widespread cultivation of Bt crops in Europe.

Keywords: *Bt* maize, western corn rootworm

Contributed Paper Tuesday, 09:45 67

Cross-resistance of Bt-resistant *Helicoverpa armigera* (Lepidoptera: Noctuidae) to *Bacillus thuringiensis* Vip3Aa protein

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The vegetative insecticidal protein *Bacillus thuringiensis* Vip3A represents a new family of Bt toxin currently applied in commercial transgenic cotton. In order to determine whether the Bt-resistant *Helicoverpa armigera* would be cross-resistant to Vip3Aa protein, insecticidal activity, proteolytic activation and binding properties of Vip3Aa toxin were conducted against Bt-susceptible strain (96S), the two Bt-resistant *H. armigera* strains which are highly resistant to Cry1Ac protein (BtR) or to Bt commercial insecticide formulation (BtI). The results showed that the resistance ratio of BtR and BtI strain were 2971.3 and 2227.0-fold resistance to Cry1Ac compared with 96S strain, only 1.7- to 3.0-fold resistance to Vip3Aa were found respectively in BtR and BtI strains than that of 96S. While digestion rate of full-length Vip3Aa with gut juice extracts from 96S was faster than that from BtR and BtI. SPR results showed binding affinity of Vip3Aa to 96S BBMV was higher than that of BtR and BtI. The results in this study indicated maybe a cross-resistance potential between Vip3Aa and Cry1Ac toxins. But because the mode of action of Vip3Aa is different form Cry1Ac, incorporating the potential effects in resistance management strategy may help to sustain the efficacy of pyramided Bt cotton.

Keywords: Vip3Aa, *Bacillus thuringiensis*, *Helicoverpa armigera*, cross-resistance

Contributed Paper Tuesday, 10:00 68

Analysis of *Manduca sexta* Aminopeptidase N1 and Alkaline Phosphatase as receptors of Cry1Ab toxin by gene silencing

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The Cry toxins, produced by *Bacillus thuringiensis*, have an outstanding interest because of their specific insecticidal activity. Their use to control pest in forest and crops is regarded as environmentally friendly. Understanding the mechanism of action of Cry toxins is a major goal to counteract the emerging resistance of insects. At least three different proteins have been proposed as Cry1A toxins receptors in *Manduca sexta*: a cadherin like receptor, a glycosylphosphatidyl inositol (GPI) anchored aminopeptidase – N (APN1) and a GPI – anchored alkaline phosphatase (ALP). In the case of the GPI-anchored receptors it has been suggested that both proteins facilitate insertion of a pre-pore oligomeric structure of Cry1Ab toxin (1). To determine the *in vivo* role of *Manduca sexta* APN1 and ALP on Cry1Ab toxicity, we are employing the RNA interference to silence the expression of APN1 and ALP and determining the toxicity of Cry1Ab on bioassays of silenced larvae. We also performed the analysis of APN and ALP enzymatic activity and their expression by rtPCR and Western blot. The results show that both receptors may have a differential participation in the mechanism of action of the Cry1Ab toxin.

1. Arenas, I., Bravo, A., Soberón M., and Gómez, I. (2010) Role of alkaline phosphatase from *Manduca sexta* in the mechanism of action of *Bacillus thuringiensis* Cry1Ab toxin. Journal of Biological Chemistry. In the press

Keywords: RNAi, Cry1Ab, Aminopeptidase N1, Alkaline Phosphatase

Contributed Paper Tuesday, 10:00 69

Oenocytoid cell lysis to release prophenoloxidase is induced by eicosanoid via PKC pathway

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Eicosanoids mediate insect cellular immune responses, which depend largely on phenoloxidase (PO) activity. In plasma, PO is activated by the proteolytic cleavage of proPO, which is stored in oenocytoids, a specific hemocyte type, of the beet armyworm, *Spodoptera exigua*. Eicosanoids induce an acute cell lysis of oenocytoids, which releases proPO into the plasma. We investigated an intracellular signal pathway following a functional interaction of eicosanoid(s) to a putative membrane receptor. U-73122 (a specific inhibitor of phospholipase C) inhibited oenocytoid lysis of *S. exigua* significantly after bacterial infection. We concluded that oenocytoid lysis required a certain level of calcium ion because EGTA (a calcium chelator) treatment inhibited cell lysis. Two protein kinase C (PKC) inhibitors (staurosporine and calphostin C) significantly inhibited the oenocytoid lysis. Oenocytoid lysis was likely induced by Na^+ entry and subsequent osmotic shock because juvenile hormone analog, pyriproxyfen, which activates Na^+-K^+ ATPase and induces subsequent cell shrinkage, antagonized the effect of eicosaniod on cell lysis. Furthermore, ouabain (a specific Na^+ pump inhibitor) significantly inhibited oenocytoid lysis. These results suggest that eicosanoid mediates oenocytoid lysis by activating the intracellular PKC pathway.

Keywords: Cell lysis; Eicosanoid; Oenocytoid; PKC; Prophenoloxidase; *Spodoptera exigua*

08:00-10:30 **TAKE POSTERS DOWN**

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

Contributed papers Tuesday, 10:30- 12:30
Hasan Turan

VIRUSES 3

Chair: Peter Krell and Primitivo Caballero

Contributed Paper Tuesday, 10:30 70

pif1 expression determines the transmissibility of the *Spodoptera frugiperda* multiple nucleopolyhedrovirus

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The Nicaraguan isolate of the *Spodoptera frugiperda* nucleopolyhedrovirus (SfNIC) survives as a complex mixture of genotypes. Each genotype in individual infections is less pathogenic than the wild-type population. Mixtures of complete genotype B (75%) and defective genotype C (SfNIC-C) (25%) restored the biological activity to that of the wild-type. Previous studies demonstrated that *pif1* and/or *pif2* genes deletion mutant genotypes were necessary and sufficient to explain the increased potency of the mixtures, but the mechanism is still unknown. In the present study two recombinant viruses were constructed with SfMNPV *pif1* gene expression reprogrammed under the SeMNPV *egt*, SfNIC-Begt, or *p10*, SfNIC-Bp10, promoters. *pif1* expression was advanced by 20 h in SfNIC-Begt infected larvae and was incresased in intensity at 120 and 144 h in SfNIC-Bp10 infected larvae, which suggests an increased production of this protein in infected cells. No significant differences were observed in final BV titres among the different viruses, however earlier BV production was observed in cells infected with the recombinant viruses. The increased proportion of PIF1 resulted in viruses 5 to 10 fold less pathogenic in terms of lethal concentration metrics, but the same viruses were more virulent, in terms of speed of kill, and consequently total OB production in Sf9 cells or *S. frugiperda* larvae was significantly reduced in recombinant viruses. Modified expression of *pif1* resulted in a 50% reduction in the relative proportion of SfNIC-Begt or SfNIC-Bp10 viruses required to achive wild-type OB potencies in mixtures with SfNIC-C. SfNIC-Begt or SfNIC-Bp10 OBs did not differ in the number of nucleocapsids/ODV or in the number of ODVs/OB from that of pure genotype B. We conclude that both OB pathogenicity and virulence are highly sensitive to the concentration of PIF1.

Keywords: *pif*1 expression, transmissibility, SfMNPV, *Spodoptera frugiperda*, mixed infections, defective genotype

Contributed Paper Tuesday, 10:45 71-STU

Baculovirus photolyases are DNA repair enzymes with circadian clock regulatory function

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The cryptochrome/photolyase family (CPF) encodes photosensitive proteins that exhibit either photoreceptor or DNA repair activity. Photolyases and cryptochromes are structurally conserved, but display distinct functions. Photolyases use visible light to repair UV-induced DNA damage. Cryptochromes, on the other hand, function as blue-light receptors, circadian photoreceptors and transcriptional repressors controlling the molecular circadian clock. Recently, we have obtained evidence that this functional divergence is not so univocal anymore. *Chrysodeixis chalcites* nucleopolyhedrovirus (ChchNPV) possesses two photolyase genes, designated *phr1* and *phr2*. It has been reported that the *phr2* gene encodes an active enzyme with DNA repair activity towards UV-induced cyclobutane pyrimidine dimers. The *phr2* gene can rescue bacteria lacking a UV repair system, whereas the *phr1* gene product does not have this ability. Here we demonstrate that PHR2 as well as PHR1, when overexpressed in NIH3T3 cells affect the amplitude of circadian oscillations, suggesting that these proteins may interact with, and thus function, in the mammalian molecular clock. Indeed, PHR2 is capable of inhibiting CLOCK/BMAL1-driven transcription of an E-box promoter containing clock genes, a function normally restricted to cryptochrome proteins. Whether

the baculoviral photolyases have a circadian role in ChchNPV-infected *C. chalcites* larvae remains to be studied This observation may be highly relevant not only for a further understanding of the evolution of cryptochrome and photolyase functions, as well as for better understanding of behavioral changes in insect virus-host pathosystems.

Keywords: CPD photolyase, cryptochrome, circadian clock, baculovirus

Contributed Paper Tuesday, 11:00 72-STU

A mechanism for AcMNPV proV-CATH retention in the endoplasmic reticulum

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The regulated and coordinated release of virus-encoded chitinase (CHIA) and cathepsin (V-CATH) enzymes at the terminal stages of baculovirus replication aids the efficient horizontal spread of occlusion derived virus. The CHIA C-terminal KDEL motif ensures endoplasmic reticulum (ER) retention of CHIA until cell lysis occurs. Like CHIA, the proenzyme form of V-CATH (proV-CATH) localizes to the ER and is also released upon cell lysis, but an ER retention mechanism for proV-CATH has not yet been identified. The *v-cath* is expressed as a preproenzyme and proV-CATH is co-translationally imported into the ER. To define the secretory signal peptide of preproV-CATH we fused either 12 or 22 codons of the *v-cath* N-terminal coding region to *gfp* to see if either peptide affords ER translocation of GFP. The 22 but not the 12 amino acid coding region of *v-cath* was sufficient to enable GFP to localize to the ER. We also wanted to determine if altering the preproV-CATH N-terminal signal peptide would alter the proV-CATH distribution (extracellular secretion vs. intracellular retention in soluble or insoluble fractions) in cell culture. For this we either substituted the putative myristic acid acceptor Gly12, to Ala12, or fused the honey bee *melittin* signal peptide coding region to either the Ile4 or Val13 codons of *v-cath*, but found that neither modification promoted changes in proV-CATH trafficking. Upon deletion of the CHIA KDEL we found that proV-CATH is co-secreted along with the ΔKDEL CHIA, suggesting a putative CHIA/proV-CATH interaction retains proV-CATH in the ER.

Keywords: Cathepsin, chitinase, AcMNPV, endoplasmic reticulum

Contributed Paper Tuesday, 11:15 73-STU

***Mamestra configurata* nucleopolyhedrovirus enhancin substrate specificity and insect intestinal mucin structural types**

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Peritrophic matrix (PM) is a non-cellular porous structure lining the insect midgut and is composed of chitin and proteins. PM serves as a barrier to insect pathogens including baculoviruses and virions must cross the PM to initiate infection of midgut epithelial cells. Some baculoviruses including *Mamestra configurata* nucleopolyhedrovirus (MacoNPV), encode metalloproteases, known as enhancins which facilitate infection by degrading insect intestinal mucins (IIMs) associated with the PM. We examined the interaction between MacoNPV enhancin and *M. configurata* IIMs both in vivo and in vitro. In vivo experiments with *M. configurata* larvae fed MacoNPV occlusion bodies (OBs) McIIM4 was degraded within 4 h post inoculation as previously demonstrated for McIIM1; however, McIIM2 was not degraded. This was further examined by in vitro experiments in which PM and MacoNPV OBs were incubated in an alkaline dissolution buffer. In addition, recombinant *Autographa californica* MNPV expressing MacoNPV enhancin (AcMNPV-enh) and wild-type AcMNPV were tested. In MacoNPV and AcMNPV-enh treatments, McIIM4 was degraded but McIIM2 was unaffected. Degradation of McIIM4 was inhibited by EDTA, a metalloprotease inhibitor, indicating that the degradation was due to enhancin. As opposed to granulovirus enhancins, MacoNPV enhancin posses a transmembrane domain at the carboxy terminus and western blots demonstrated that enhancin was present in the ODVs of AcMNPV-enh and MacoNPV. MacoNPV enhancin substrate specificity and the phylogeny of different classes of IIMs suggest that MacoNPV enhancin is capable of degrading complex IIMs (McIIM1 and McIIM4) but not binary IIMs (McIIM2).

Keywords: Peritrophic Matrix, insect intestinal mucin, enhancin, *Mamestra configurata*

Contributed Paper Tuesday, 11:30 74

N-terminal of VP3 of *Dendrolimus punctatus* cypovirus plays an essential role in attaching to its host cells

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Cytoplasmic polyhedrosis viruses (CPVs) belonging to the *cypovirus* genus within the *Reoviridae* family typically have genomes comprised of 10-11 dsRNA segments. *Dendrolimus punctatus* CPV (DpCPV) is an important pathogen of the pine caterpillars and has been developed as a commercial insecticide in China. The completed sequence of DpCPV genome has been determined in our laboratory. However, little is known about the mechanism of its infection. In current study, the full-length or partial fragments of the genomic segment 1, segment 4, segment 6 and segment 7 of DpCPV, which encode the structural proteins, are reversely transcribed and expressed in *E. coli*. Cell-binding assay using flow cytometry indicates that the binding intensity of theN-terminal of VP3, encoded by 3' 1200 bp of the segment 4, to the Sf9 cells is significantly higher than the other expressions. Immunization block experiments indicate that the antibody against the VP3 N-terminal is able to significantly lower the infectivity of DpCPV virions both *in vitro* and *in vivo*. Ligand-blotting experiment demonstrates that the VP3 N-terminal recognizes a 28 kD molecule on the brush-border membrane vesicles

of *Spodoptera exigua* larvae. In conclusion, the VP3 N-terminal of DpCPV might take charge of the attaching role during its infection process.

Keywords: *Dendrolimus punctatus* cypovirus, VP3, Attachment protein

Contributed Paper Tuesday, 11:45 75

Genome sequence of a granulovirus occlusion body shape/size mutant

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An unusual granulovirus was isolated from Adoxophyes honmai larvae and characterized. The occlusion body (OB) was cuboidal and 0.5-2 mm in diameter, but contained only one virion. This newly isolated virus was classified as a granulovirus because the sequences of several genes, including granulin, and of the major structural protein were closely related to those of A. orana granulovirus (AdorGV). We designated this virus the AdorGV-OB mutant (OB mutant). OBs of AdorGV (normal strain) were ovicylindrical and approximately 0.5 mm in diameter. The OB mutant genome is 99,507 bp and has 122 putative ORFs; the normal strain is 99,657 bp and has 119 putative ORFs (Wormleaton et al., 2003). The two genomes were highly similar: 66 ORFs of the OB mutant were 100% identical and 53 were more than 90% identical to their homologs in the normal strain. Three OB mutant ORFs, whose functions were unknown, displayed less than 90% identity with their normal strain counterparts. Three others, encoding the structural proteins ODV-E66 and vp91-capsid, and ORF112 (function unknown), possessed internal stop codons that divided them into two ORFs. Are larger OBs more UV-tolerant? The virulence of OBs from the OB mutant and normal strain exposed to UV light was compared. Significantly more A. honmai larvae were killed after inoculation with UV-exposed OBs of the OB mutant than of the normal strain.

Keywords: Granulovirus / occlusion body / genome / UV-tolerance

Contributed Paper Tuesday, 12:00 76

Formation of few polyhedra (FP) by AcMNPV 25K FP mutants is dependent on cell lines

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Development of few polyhedra (FP) phenotype of baculovirus in cell lines was reported due to mutations of the *25K FP* gene in several nucleopolyhedroviruses (NPVs). The *25K FP* gene has been identified involved in membrane protein trafficking in the nucleus where NPV replicates. Inactivation of NPV *25 K FP* during passage in cell culture leads to reduced polyhedral production in cells, increased budded virus (BV) production, and reduced virulence of NPV in insects by *per os* feeding of polyhedra. In a plaque assay of wild type *Autographa californica* MNPV (AcMNPV) in Sf21 cells, two AcMNPV plaques were identified by PCR amplification of the *25K FP* gene using an pair of AcMPV *25K FP* primers with an expected 1.2 kb DNA product. One plaque (AcP13-2) showed a 1.5 kb product with a 0.3 kb DNA insertion in the *25K FP* gene and the other plaque (AcP2) showed a 1.2 kb product with site mutations. We found AcP13-2 and AcP2 formed FP in High 5 cells derived from *Trichoplusia ni* but production of polyhedra of AcP13-2 and AcP3 in Sf21 cells is indistinguishable from that of AcMNPV plaque (AcP3) that has a functional *25K FP* and produces multiple polyhedra (MP) in High 5 cells. Comparison of polyhedron production yields of AcP13-2 and AcP3 in Sf21 cells showed no difference. In both AcP13-2 and AcP3, some Sf21 cells showed MP and others showed FP. The numbers of polyehdra/cell in Sf21 by both AcP13-2 and AcP3 are correlated with the sizes of the cells.

Keywords: AcMNPV, FP, 25k FP

Contributed Paper Tuesday, 12:15 77

A silencing suppressor protein (NSs) of a tospovirus enhances baculovirus replication in permissive, semipermissive and nonpermissive insect cell lines

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The nonstructural protein (NSs) of the *Tomato spotted wilt virus* (TSWV) has been identified as an RNAi suppressor in plant cells. A recombinant *Autographa californica nucleopolyhedrovirus* (AcMNPV) denominated vAcNSs, containing the NSs gene under the control of the viral polyhedrin (*polh*) gene promoter, was constructed and the effect of NSs in permissive, semipermissive and nonpermissive insect cells to vAcNSs infection was evaluated. We showed that vAcNSs produced more budded virus when compared to wild type in semipermissive cells. Furthermore, vAcNSs caused cytopathic effects in a nonpermissive cell line whereas infection with wild type AcMNPV showed no cytopathic effects. Co-infection of vAcNSs with wild type baculoviruses clearly enhanced polyhedra production in all host cells. Confocal microscopy analysis showed that NSs accumulated in abundance in the cytoplasm of permissive and semipermissive cells. In contrast, high amounts of NSs were detected in the nucleus of nonpermissive cells. Co-infection of vAcNSs with a recombinant AcMNPV containing the enhanced green fluorescent protein (EGFP) strongly increased GFP expression in semipermissive cells and in *Anticarsia gemmatalis*-hemocytes. Absence of small RNA molecules of *egfp* transcripts in this cell line and in a permissive cell line indicates the suppression of gene silencing activity. On the other hand, vAcNSs was not able to suppress RNAi in a nonpermissive cell line. The NSs-nuclear localization suggested a novel function of this protein allowing AcMNPV late gene expression in nonpermissive cells. Our data supported that NSs protein of TSWV facilitates baculovirus gene expression in different insect cells lines.

Keywords: Baculovirus, NSs, Tospovirus, RNAi, host range

Contributed papers
Tuesday, 10:30-12:30
Fahri Kuran

FUNGI 2

Chairs: Jarrod Leland and Ismail Karaca

Contributed Paper Tuesday, 10:30 78

Mechanism of long-term suppression of a forest pest by application of *Beauveria bassiana*

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Long-term suppression of the populations of the Masson's pine caterpillar, *Dendrolimus punctatus*, can be achieved by inoculative release of *Beauveria bassiana* as compared to inundative release. A 10-year study revealed the mechanism of the suppression as follows: Compared to inundative release, inoculative release less disturbs ecosystem, suggesting that long-term suppression is associated to species diversity. Genetic diversity of *B. bassiana* population is very abundant in the forest community, forming quite a few host chains. When host population is low, the artificially released strain can find proper hosts to survive by host transfer. Niche overlap of different strains happens on some insects. When the released strain is at low level, some other strains can also suppress the caterpillars through host transfer as another density dependent factor. In this case, *B. bassiana* displays a natural multiple mortality factor strategy, instead of just a single factor strategy. It also suggests that the long-term suppression is associated to genetic diversity of *B. bassiana*, and genetic diversity works based on species diversity. The population of *B. bassiana* is highly heterogenous, making it one of the keystone species in maintenance of insect community stability in the pine ecosystem. Genetic exchange and recombination occurs frequently between different strains of *B. bassiana*, resulting in heterokaryons to maintain the genetic diversity. Based on the above mechanisms the commonly used inundative application strategy of fungal insecticides for forest pest control should be substituted by a long-term inoculative application strategy to fully take advantage of natural epizootics.

Keywords: Inoculation biological control, inundation biological control, host transfer, niche overlap, keystone species

Contributed Paper Tuesday, 10:45 79

Transmission of *Metarhizium anisopliae* between male and female Asian longhorned beetles, *Anoplophora glabripennis*

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Asian longhorned beetle (*Anoplophora glabripennis*) adults inoculated with *Metarhizium anisopliae* transmitted conidia to mates during laboratory bioassays. Six hours after inoculation and placement with a mate, conidia washed from beetles using Tween-80 and pentane were quantified to determine that males had transmitted more conidia to females than females had transmitted to males. During bioassays, the individual inoculated with conidia always died more quickly than its mate after male and female were caged together. Bioassays also demonstrated a smaller difference between time to death for males and females when males were contaminated with conidia than when females were contaminated with conidia. We hypothesize that the greater transmission of spores from males to females than from females to males is due to a combination of the "top" position of males during copulation and the prolonged mate guarding behavior of male *A. glabripennis*, during which males remain 'mounted' on females for prolonged periods.

Keywords: Transmission, entomopathogenic fungi, microbial control, mate guarding, Asian longhorned beetle, *Metarhizium anisopliae*

Contributed Paper Tuesday, 11:00 80

Occurrence of entomopathogenic fungi in soils from different habitats in Poland

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Entomopathogenic fungi are a group of microorganisms important for regulation of arthropod populations both in natural and managed ecosystems. The purpose of this study was to investigate diversity and occurrence of entomopathogenic fungi in the soil from different habitats in agricultural landscape. A total 296 soil samples from 74 locations in Poland were collected from different habitats, mainly agrocenoses (arable fields, meadows, pastures), semi-natural (shelterbelts, field afforestations, balks) and forest biotopes. Insect-pathogenic fungi were isolated from soil samples by means of the "Galleria bait method". In the soils from agrocenoses and semi-natural habitats in Poland, eight entomopothogenic fungal species: *Beauveria bassiana*, *B. brongniartii*, *Isaria farinosa*, *I. fumosorosea*, *Metarhizium anisopliae*, *M. flavoviride*, *Lecanicillium sp*. and *Conidiobolus coronatus* were found. Generally, three fungi *B. bassiana* (isolated from 74,4% soil samples), *M. anisopliae* (69,3%) and *I. fumosorosea* (68,6%), dominated in Polish soils. The dominance of particular species depended on habitat type. In soils from arable fields *M. anisopliae* and subsequently *I. fumosorosea* and *B. bassiana* the most frequently occurred. *M. anisopliae*, *B. bassiana* and then *I. fumosorosea* were dominant in the soils from meadows and pastures. In the soil and litter samples from forest habitats fungus *B. bassiana* decidedly dominated. *B. bassiana* was most frequently isolated from the forest litter than soil, but the forest soil was more rich in fungal species. Eight insect-pathogenic fungi were isolated from the soils of semi-natural habitats. The most frequently *I. fumosorosea* and *B. bassiana* occurred. Semi-natural habitats, especially shelterbelts, are characterized by more abundant fungal species composition, being peculiar refuges for their resources, diversity and persistence in the agroecosystem.

Keywords: Soil, entomopathogenic fungi, insect bait method, habitat

Contributed Paper Tuesday, 11:15 81

Identification, isolation and virulence of entomopathogenic fungi collected from cereal aphids in Argentina and South Africa

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Field surveys were conducted in Argentina during October/November 2008 and 24 isolates of *Pandora neoaphidis* (Entomophthorales) were collected. The majority of cereal aphid hosts harbouring *P. neoaphidis* were alates of *Metopolophium dirhodum*, and *Sipha maydis*. *Entomophthora planchoniana* (Entomophthorales) and the two hyphomycetous fungi *Beauveria bassiana* and *Isaria fumosorosea* were also recorded. Surveys conducted in South African wheat during November 2009 (Bethlehem, Free State province) recorded two entomophthoralean fungi, *viz*., *P. neoaphidis* and *E. planchoniana*. Aphid hosts included *Diuraphis noxia*, *Sitobion avenae* and *M. dirhodum.*
Bioassays were conducted to compare the performance of an Argentinean (ARG 26) versus South African (SGI 897) strain of *B. bassiana* against *D. noxia* and *S. avenae*. Suspensions containing 3.8 x 10^7 conidia per ml (+ 0.01% BreakThru surfactant) were prepared and 5 ml aliquots sprayed through a Burgerjon spray tower. Doses administered per mm^2 with each of the two fungi were 802±57 and 781±13 conidia, respectively. Five groups of 20 aphids per group were sprayed (total 300 aphids of each species; including controls) with each of the two fungal isolates. The assay protocol employed showed a higher level of suitability toward *D. noxia* (control mortality = 8%) compared to *S. avenae* (control mortality = 37%). Data gathered with *D. noxia* was therefore more reputable with (cumulative) mortalities ranging from 11% (ARG 26) to 25% (SGI 897) on day 6. However, the highest level of overt mycosis was 65% recorded with ARG 26 on *S. avenae* with levels ranging from 42 – 45% for the other three treatments. This observation suggests that ARG 26 may be better adapted to *S. avenae* than any of the other pathogen-host combinations.

Keywords: Aphids, cereals, Argentina, Southafrica, entomopathogenic fungi

Contributed Paper Tuesday, 11:30 82

Culture of *Metarhizium robertsii* on salicylic-acid supplemented media induces increased conidial thermotolerance, but not UV-B tolerance

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Salicylic acid (SA), a cell signaling metabolite in plants, is involved in their resistance to plant pathogens and environmental stresses; but there is little information available on the responses of fungi to SA. We have examined the possibility that conidia of an entomopathogenic fungus (EF) produced on medium with salicylic acid will have enhanced tolerance to stress, particularly to UV-B radiation and heat. Conidia of the EF *Metarhizium robertsii* (ARSEF 2575) were produced on potato dextrose agar plus yeast extract medium (PDAY) supplemented with 1, 2, 4, and 8 mM SA (pH adjusted to 6.9) under dark conditions. For comparison, conidia also were produced on minimal medium (MM) (carbon starvation) under continuous-dark incubation, a condition known to induce in *M. robertsii* elevated conidial tolerance to heat and UV-B radiation. The heat tolerances of conidia produced on PDAY medium containing 1, 2, and 4 mM SA were two-fold higher than that of conidia produced on PDAY alone. The thermotolerance of these SA-produced conidia was not different from that of conidia produced on MM. Conidia produced on PDAY with 8 mM SA did not exhibit elevated heat tolerance. Growth on SA did not increase conidial UV-B tolerance. The conidial yields of *M. robertsii* produced on PDAY medium with all levels of SA were reduced in comparison to yield on PDAY alone. Nevertheless, conidial yields with SA were higher than yields obtained on minimal medium alone. In conclusion, *M. robertsii* conidia produced on medium containing low concentrations of SA demonstrated improved tolerance to heat, but not to UV-B radiation

Keywords: Salicylic acid, *Metarhizium robertsii*, entomopathogenic fungus, UV-B tolerance, thermotolerance, cross resistance

Contributed Paper Tuesday, 11:45 83

Effect of temperature and time of exposure on the viability and virulence of *Beauveria bassiana* and *Metarhizium anisopliae* in dried unformulated Conidia and formulated Conidia

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The influence of temperatures and time of exposure on viability and virulence of suspensions of *Beauveria bassiana* and *Metarhizium anisopliae* were evaluated under laboratory conditions. The fungi were used as pure conidia, Rice plus fungus, in wettable powder and emulsifiable oil formulation. Conidia suspensions were maintained at 26°C, 36°C and 46°C for 1h, 4h and 6h. Four independent suspensions (repetitions) were prepared in each experiment which were placed in centrifuge tubes and incubated under the respective temperatures during each period. Aliquots of suspensions were then plated to determine viability, and were also sprayed onto larvae of *Diatraea saccharalis* in order to evaluate the fungi virulence. In another experiment, the effects of temperature on the viability of pure dry conidia and formulated conidia in emulsifiable oil were compared by incubating both fungi at 26°C, 36°C and 46°C for up to 12 hours at 30 minutes intervals. In general, increase in temperature negatively affected the germination and fungi efficiency, and this relationship was proportional to the duration of exposure to temperature. The emulsifiable oil formulation was the least affected by the detrimental effects of heat for both fungi. Even after 6 hours of exposure to temperature of 36°C, the

viability remained above 85% for both species evaluated. This formulation also caused higher insect mortality, and even after 4h of exposure to 46°C the efficiency was 39% and 50% for *B. bassiana M. anisopliae*, respectively. *M. anisopliae* was more sensitive to the effect of temperature than *B. bassiana*, presenting greater reductions on the conidial viability (after 12h of exposure to 36 ° C the viability of pure conidia was reduced by 13% compared to 5% reduction in *B. bassiana*). The results showed that the formulation in emulsifiable oil of both fungi is recommended for control of *D. saccharalis*, because it protects the fungi from the adverse effects of temperature resulting in higher mortality than other forms of fungi tested.

Keywords: *Diatraea saccharalis*, Entomopathogenic fungi, Oil formulation, Wettable powder

Contributed Paper Tuesday, 12:00 84

Effect of light intensity and time of exposure on sporulation and germination of *Neozygites floridana*

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Neozygites floridana is a cosmopolitan pathogen of spider mites and frequently causes natural epizootics on *Tetranychus* spp. Light has been reported to inhibit sporulation while darkness is thought to promote sporulation of *N. floridana*. However, the effect of intensity and duration of light exposure has not been studied. This study determined the effect of light intensity and duration of exposure on sporulation and germination of isolates of *N. floridana* from Norway and Brazil. The two countries have different photoperiods during the seasons when epizootics of this fungus are observed. The experiment was conducted by placing individual cadavers of each isolate (Brazil and Norway) on three square photo-etched coverslips (18 x18 mm) inside six transparent plastic chambers lined with wet filter papers to reach 100% relative humidity and sporulation and germination quantified under the microscope. The treatments for each isolate consisted of two light intensities, 40 and 208 µmol $m^{-2}S^{-1}$, two temperatures, 18 and 23°C and three photoperiods, 24h of continuous light, 12h darkness preceded by 12h light and 24h continuous darkness (control). The results indicate that continuous full light for 24h inhibits sporulation and germination for both isolates at both temperatures. 12h of darkness preceded by 12h light produces similar results of sporulation with 24h continuous darkness (control). However, germination inhibition by light was stronger on the isolate from Norway than Brazil. Dim continuous light for 24h had a mild effect on sporulation on both isolates but strongly inhibited germination of the Norwegian isolate. The results suggest that light intensity and duration (photoperiod) may play an important role on the field epizootiology of *N. floridana*.

Keywords: Spider mites, Photoperiod, Entomopathogens, Epizootics, *Tetranychus* spp., Biological control

Contributed Paper Tuesday, 12:15 85-STU

Cold-seeking behaviour in *Drosophila* during mycosis: Consequences for host and pathogen

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Behavioural changes of temperature preference in response to an infection have been well documented across a range of taxa. Often these have been associated with a direct detrimental effect on the pathogen and an overall improvement to other aspects of the immune response. Here, we show that the fruit fly, *Drosophila melanogaster*, responds to infection by the fungal pathogen, *Metarhizium anisopliae*, by seeking out colder temperatures. We report the fitness consequences of this behaviour for both pathogen and host. Although, cold-seeking behaviour is detrimental for the pathogen, there were no benefits of cold-seeking behaviour for the host. We discuss the implications of the results for our understanding of host/pathogen coevolution.

Keywords: cold-seeking (anapyrexia), Drosophila, Metarhizium,fecundity, longevity

Contributed papers
Tuesday, 10:30-12:30
Nihat Turan 1

MICROBIAL CONTROL 2

Chairs: Jean Maniania and Reza Talaei

Contributed Paper Tuesday, 10:30 86-STU

Effects of the presence of susceptible and non-susceptible insects on *Beauveria bassiana* F418 *gfp* tr3 persistence in soil

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Sitona lepidus Gyllenhal (Coleoptera: Curculionidae) is a major pest of white clover in New Zealand pastures. Although effective in the laboratory, the entomopathogenic fungus *Beauveria bassiana* F418 has provided variable control of *S. lepidus* in the field. A better understanding of the ecology of the pathogen in soil is needed to enhance efficacy. Using a transformed strain of the fungus expressing the green fluorescent protein and the hygromycin B resistance gene (F418 *gfp* tr3), effects of a susceptible (*Tenebrio molitor* L., Coleoptera: Tenebrionidae) and non-susceptible (*Costelytra zealandica*, Coleoptera: Scarabaeidae) insect on F418 *gfp* tr3 persistence in pasteurised and non-sterile soils was assessed. The

F418 *gfp* tr3 population increased significantly in the presence of *T. molitor* and decreased in the presence of *C. zealandica.* Accompanying studies demonstrated that F418 *gfp* tr3 conidia germinated on the cuticle of *C. zealandica* but infection does not occur, leading to a net loss of viable conidia from the soil. Conidia administrated orally to *C. zealandica* were recovered in faecal samples, suggesting that ingestion of the fungus by the insects has little impact on the viable soil population. This study demonstrates that non-susceptible insects can reduce inoculum loadings in soil over time.

Keywords: *Beauveria bassiana*, persistence, non-susceptible insects, germination

Contributed Paper Tuesday, 10:45 87-STU

Establishment of the fungal entomopathogen *Beauveria bassiana* (Ascomycota: Hypocreales) as an endophyte in broad bean and oilseed rape and its potential for insect biocontrol

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The ability of fourteen strains of the fungal entomopathogen *Beauveria bassiana* (Balsamo) Vuillemin to endophytically colonize broad bean *Vicia faba* L. (Fabaceae) and oilseed rape *Brassica napus* L. (Brassicaceae) after being artificially introduced into plants was investigated. Twelve of the tested *B. bassiana* strains were able to colonize inoculated leaves of both host plants after foliar spray. However, percent colonization varied significantly among strains within each host plant. The virulence of the twelve endophytic *B. bassiana* strains against *Helicoverpa armigera* Hubner on *V. faba* was investigated in a subsequent experiment in order to examine their potential as biocontrol agents against this insect pest. Although all the endophytic *B. bassiana* strains significantly affected the survival of third instar *H. armigera*, larvae fed among plants inoculated with different strains showed significant differences in percent mycosis and survival time of *H. armigera*. This study reports for the first time the endophytic establishment of *B. bassiana* in *V. faba* and *B. napus*, and adds to the host breadth of this fungus as an endophyte. It also demonstrates the exciting potential of using endophytic *B. bassiana* as a biocontrol agent, a field that definitely merits further investigation.

Keywords: *Beauveria bassiana*, *Brassica napus*, biocontrol agent, entomopathogenic fungi, fungal endophytes, *Helicoverpa armigera*, host range, strain variability, *Vicia faba*

Contributed Paper Tuesday, 11:00 88

Effect of soil moisture and texture on virulence of entomopathogenic fungi

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Soil-borne pests are important agricultural pests that their chemical control not only is less effective but also is hazardous for soil ecosystem. Soil environment is a rich source of Entomopathogenic fungi (EPF) that play an important role in controlling of agricultural pests. There are several abiotic factors which affect on persistence and virulence of EPF in soil. In this study, effect of moisture and texture of soil on virulence of two fungal species, *Metarhizium anisopliae* and *Beauveria bassiana* isolated from soil was investigated. Moisture treatments were 5, 10 and 15 % w/w moisture and texture treatments were sandy-loam and clay. Spore suspension of 10^7 spores/g against *Galleria mellonella* was used. Results showed that moisture and texture of soil had significant effect on virulence of both species. Soil texture had significant effect on mycosis and *M. anisopliae* caused more larval mortality. The results of this study can be used in selecting proper isolates for soils with different moisture content and texture.

Keywords: Entomopathogenic fungi, soil, moisture, texture, virulence

Contributed Paper Tuesday, 11:15 89

Field efficacy of the *Metarhizium anisopliae* isolate ICIPE78 in controlling the red spider mite *Tetranychus evansi* in tomato field crop in Central Kenya

David Mogisho Bugeme[1]; **Nguya Kalemba Maniania[1]**; **Adenirin Chabi-Olaye[1]**; **Hamadi Iddi Boga[2]**; **Markus Knapp[1]**
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The tomato red spider mite, *Tetranychus evansi*, is an invasive species from South America introduced into Africa in the 70s. It has become a serious pest and a major constraint to tomato production in many parts of Africa. *T. evansi* also causes extensive damage to other vegetable crops. African farmers rely almost exclusively on frequent applications of highly toxic acaricides to control this pest which leads to increase mite reproduction and development of resistance. The need to develop alternative control strategies is critical considering the cost, decreasing efficacy and potential human and environmental hazards. Classical biological control through introduction of predatory mite and fungal pathogen *Neozygites floridana*, and the use of mitosporic entomopathogenic fungi in inundative approach are among the alternatives being considered. The efficacy of aqueous and emulsifiable formulations of the *Metarhizium anisopliae* isolate ICIPE78 was evaluated against *T. evansi* on tomato plants in the field in two-season experiments in Kenya. Abamectin, a synthetic acaricide was included as a check. Tomato plants were artificially infested with mites and allowed to establish and multiply. Treatments were applied weekly until harvest. The pest density was lower in aqueous and emulsifiable formulations and abamectin treatments than in the controls in both seasons. However, there were no significant differences in the number of tomato fruits/plant in both seasons between the treatments. Metric ton of tomato per ha was not significant between the treatments in the first season but was

higher in the treated plots than and in the controls in the second season.

Keywords: Fungal formulations, Tomato crop, *Metarhizium anisopliae*, *Tetranychus evansi*, Microbial control, Acaricide

Contributed Paper Tuesday, 11:30 90

Can *Metarhizium anisopliae* treated semiochemical-baited traps reduce *Amblyomma variegatum* populations in the field?

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Amblyomma variegatum is the most widely distributed tick species across the African continent and was recently introduced into the Caribbean. It transmits *Ehrlichia ruminantium* and *Dermatophilus congolensis* in cattle, and also a vector of *Rickettsia africae* which is pathogenic to humans. The infestation can cause considerable losses in the livestock industry. The use of entomopathogenic fungi is one of the alternatives being considered for their control. We evaluate the performance of *Metarhizium anisopliae*-treated semiochemical-bait traps in reducing *A. variegatum* populations in the field. An emulsifiable formulation of *M. anisopliae* was applied in semiochemical-baited traps placed at five spots within 100-m plot and treatments were repeated after 14 and 28 days soon after rotating the traps clockwise in order to cover different sections of the plot. Five untreated semiochemical-baited traps were deployed in each plot for 3 successive days 6 weeks post-treatment to trap surviving ticks. The percentage of ticks recovered in the fungus-treated plots was significantly lower (31.1%) than in the control plots (85.6%), representing a relative tick reduction of 63.7%. Mortality of 93.83% was observed among the ticks that were recovered from the field and maintained in the lab. Some of the surviving ticks were evaluated for their reproduction potential. Fungus-infected female ticks had a longer period of engorgement, 17 days compared to 14 days in the control, and ingested low amount of blood. Pre-oviposition period took longer in fungus-treated than in control females, and the weight of egg masses was less than the ones laid by control females.

Keywords: Tick, *Amblyomma variegatum*, *Metarhizium anisopliae*, semiochemical, trap, control

Contributed Paper Tuesday, 11:45 91

Synergistic effect of dual imidacloprid *Metarhizium anisopliae* applications against asian longhorned beetles (*Anoplophora glabripennis*)

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Anoplophora glabripennis (Motschulsky) (Coleoptera: Cerambycidae), a longhorned beetle species native to Asia has been introduced into several North American and European cities. Currently eradication and preventive measures are limited to identifying and destroying infested trees and protecting uninfested trees with trunk or soil-injections of the systemic insecticides imidacloprid. Because entomopathogenic fungi like *Metarhizium anisopliae* (Metsch.) Sorokin have been identified as virulent against these beetles we conducted several tests to determine the compatibility of the two agents in combination. In a 2x3 factorial experiment investigating potential interactions between exposure to imidacloprid and *M. anisopliae* we observed no effect of imidacloprid alone on beetle survival at a single dose of 10 or 100 ppm compared to control insects, a significant effect of exposure to *M. anisopliae*, and a significant interaction between imidacloprid and *M. anisopliae* representing a synergistic (not additive) effect of dual treatment. Beetles exposed to the fungus alone lived significantly longer compared to insects treated with a single dose of 100 ppm imidacloprid (9.5 vs. 6.5 days). Consumption of twigs by beetles exposed to imidacloprid and *M. anisopliae* in a factorial experiment revealed a significant reduction in consumption (48% and 16%) over the 6-day test period as a function of exposure to *M. anisopliae* and imidacloprid, respectively. Beetles fed100 ppm imidacloprid consumed 32% less over the first three days compared to beetles not exposed to imidacloprid and thereafter consumed as much as beetles not fed 100 ppm imidacloprid, whereas *M. anisopliae*-exposed beetles consumed significantly less food throughout the test period.

Keywords: Asian lonhorned beetle, imidacloprid, *Metarhizium anisopliae*

Contributed Paper Tuesday, 12:00 92

Origin and spreading track of white muscardine of silkworms

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White muscardine disease of silkworms is an important influencing factor of sericulture. It is essential for muscardine control to reveal the origin and its spreading track of a pathogenic strain. 70 isolates of *Beauveria bassiana* were obtained from silkworm and some other insects in rearing rooms, and ground, walls, rearing facility inside the rooms, and soil from surrounding mulberry garden, cropland, and adjacent pine plantation. ISSR was used to analyze them and 6 other isolates of *B. bassiana* for tracing origin and spreading track of silkworm pathogens. The results of UPGMA cluster analysis and 3-d principal coordinate analysis both revealed that the local population of *B. bassiana* was heterogenous with obvious dominance. The predominant group prevailed in silkworms and some alternate coleopetran hosts in surrounding forest and cropland and contaminated rearing room environment and facility, and soil of surrounding mulberry orchard, pine plantation and crop field. Another group from a leafroller, an ant in pine plantation and another lepidopteran species in the cropland also caused the muscardine, and was also

detected in cropland soil and rearing facility. The 3rd group causing dramatic epizootic of mantids around rearing rooms was not associated with the silkworm muscardine. The 4th group consisting of 2 production strains and 4 indigenous strains from surrounding pine caterpillars was genetically far. Bioassay showed that the silkworm-pathogenic isolate was highly virulent on silkworms while the production strain poorly virulent, suggesting that either fungal insecticide application against pine caterpillars and pine sawyers or indigenous natural enizootics is safe on sericulture.

Keywords: *Beauveria bassiana*, epizootic, biological control, molecular marker, ISSR

Contributed Paper Tuesday, 12:15 93

Nuclear Polyhedrosis Virus (NPV) infection counters insecticide resistance in *Helicoverpa armigera*

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The evolution of insecticide resistance in *Helicoverpa armigera* is a major threat to the effectiveness and sustainability insect pest programs in various parts of the world and therefore affects agriculture economy of several countries including Pakistan. Through their unique mode of action, entomopathogenic virus provides promising alternatives to chemical control. However, potential interactions between virus infection and insecticide resistance, such as cross-resistance, have not been investigated in this important pest of several crops. We show that insecticide-resistant *H. armigera* remain susceptible to infection with the virus, nuclear polyherdrosis virus (NPV). Two different strains of *H.* armigera selected with spinosad (SPIN-SEL) and deltamethrin (Delta-SEL) were equally susceptible to NPV infection as their baseline counterparts, showing significantly reduced survival. Moreover, NPV infection reduced the expression of resistance to spinosad and deltamethrin. *Helicoverpa armigera* preinfected with NPV showed a significant increase in mortality after insecticide exposure compared with uninfected control. Our results show a high potential utility of NPV based biopesticides for existing control measures and provide products for use in resistance management strategies.

Keywords: NPV, *Helicoverpa armigera*, insecticides Resistance

Contributed Paper Tuesday, 12:30 94

Baculovirus - How much is a lethal concentration?

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Baculoviruses are becoming more widely used worldwide for control of lepidopteran pests of agricultural crops. Three baculoviruses are registered for use in South Africa. These are the *Helicoverpa armigera* nucleopolyhedrovirus (HearNPV) against bollworm on various crops, the *Cydia pomonella* granulovirus (CpGV) against codling moth on apples and the *Cryptophlebia leucotreta* granulovirus (CrleGV) against false codling moth (FCM) on citrus and avocadoes. It is generally accepted that unlike chemical insecticides, there is no linear dose-response to baculovirus concentration in field usage above a critical minimum dosage of virus particles. Efficacy of a spray is therefore a factor of the statistical probability of larvae ingesting a lethal concentration of virus. A model is being developed to relate concentration of CrleGV applied, to degree of control of FCM on citrus. The model relates virus density on the fruit surface to neonate larval movement. It considers the mean lethal concentration of virus for neonate larvae and the probability of a larva encountering and ingesting such a concentration. Differences in efficacy of different virus concentrations applied, can therefore be estimated. Results of field trials of two different commercial formulations of CrleGV applied at different concentrations are reported. This allows comparison between the model and applied usage of CrleGV. Ultimately, calculation of the correct virus concentration for an acceptable level of efficacy in the field should be possible.

Keywords: *Cryptophlebia leucotreta* granulovirus, citrus, model

Contributed papers
Tuesday, 10:30-12:30
Nihat Turan 2

MICROSPORIDIA 1

Chairs: David Oi and Kubilay Er

Contributed Paper Tuesday, 10:30 95

Rapid build up of *Nosema fumiferanae* in outbreak populations of the jackpine budworm, *Choristoneura pinus pinus*

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Nosema fumiferanae is the most ubiquitous pathogen that has been implicated as a regulatory factor in populations of the spruce budworm, *Choristoneura fumiferana*, but typically requires years of high-density populations to reach high prevalence levels. Because outbreak densities of the closely related jack pine budworm, *Choristoneura pinus pinus*, persist for only 2-4 years, *Nosema* may not be able to build up in those populations unless transmission is much more efficient than in the spruce budworm-fir-spruce system. We monitored its prevalence during the course of the most recent outbreak in Ontario, Canada, through microscopic examination of ~15,000 overwintering (second-instar) larvae. Monitoring between 2004 and 2009 revealed a rapid build up of *Nosema* in outbreak populations of jack pine budworm. Rapid build up was evident from changes in regional- and district- wide prevalence as the outbreak progressed, an increase in the proportion of monitored sites that was infected, and an average 10- to 13-fold increase in % infected larvae from one year to the next in sample plots that were monitored for two consecutive years. We postulate that transmission of

Nosema in jack pine budworm is more efficient relative to spruce budworm because the life cycle of this species presents two opportunities for horizontal transmission: during the pollen cone-feeding stage of early instars and during the shoot-feeding stage of late instars, while larva-to-larva transmission in spruce budworm populations is limited to the shoot-feeding stage of late instars. A possible role of *Nosema* in jack pine budworm outbreak dynamics should be considered.

Keywords: *Nosema fumiferanae*, Choristoneura, transmission

Contributed Paper Tuesday, 10:45 96

Occurrence of the microsporidium *Canningia tomici* and artificial infection in the pine shoot beetles *Tomicus piniperda* and *Tomicus minor* (Coleoptera, Scolytidae)

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The pine shoot beetles *Tomicus piniperda* and *Tomicus minor* are major tree pests, particularly of *Pinus sylvestris* but also of *Pinus nigra* depending on geographical region. *T. piniperda* is found throughout Europe, from the far north to the south of central Europe, and *T. minor* is found all over Europe. *T. piniperda* has spread to China and has been introduced into North America.
The occurrence and prevalence of the microsporidium, *Canningia tomici*, described from *T. piniperda* was investigated by dissection of adult beetles from different regions in Europe and from Canada. In addition, infection experiments were conducted with *C. tomici* in the laboratory. *C. tomici* caused infection in *T. piniperda* and *T. minor*, surprisingly in higher infection rates in the latter species. Differences were found in infection rates of parental and offspring beetles and also depending on incubation temperature.

Keywords: Canningia, Tomicus

Contributed Paper Tuesday, 11:00 97

Molecular phylogenetics of *Thelohania muelleri* like parasites infecting gammarid amphipods

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Thelohania muelleri is a well described Microsporidian found infecting the musculature of gammarid amphipods. It produces a distinctive pathology, consisting of white masses of spores visible in the abdomen of the host, which is commonly used as the diagnostic feature when identifying the infection. Difficulty in obtaining molecular data has resulted in the species only previously being characterised ultrastructurally. 16S rDNA sequence was acquired from individuals of *Gammarus lacustris, G. duebeni* and *G. fossarum* all showing the typical pathology associated with *Thelohania muelleri*. Phylogenetic analysis by Bayesian Inference suggests that the species is actually a member of the genus *Dictyocoela*. A formal description of *Dictyocoela* has yet to be published. However, previous studies associate *Dictyocoela duebenum* with light infections of the gonad and ectodermal tissues, with no mention of the gross muscle pathology associated with *T. muelleri*. Therefore to obtain a true full description, infection in the musculature must also be investigated. It is possible that if *Thelohania muelleri* is in fact a *Dictyocoela* parasite that other morphologically similar species such as *T. hereditaria* should also be transferred to the genus.

Keywords: *Thelohania muelleri*, microsporidia gammarus phylogeny

Contributed Paper Tuesday, 11:15 98

Molecular phylogeny of five microsporidian species infecting *Chironomus plumosus* (Diptera: Chironomidae) in North-Western Russia

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Midge larvae (family Chironomidae) are among the most preferred insect hosts of microsporidia that can be explained by their long-term development and mass propagation in silt layer at lake bottoms, favorable for acquirement of microsporidian spores from various sources. Over 50 species of microsporidia have been described from chironomids, and rDNA sequences were acquired for five species recently, namely *Semenovaia chironomi, Neoperezia chironomi, Helmichia lacustris, Anisofilariata chironomi* and *Microsporidium sp.,* all sampled in vicinities of St. Petersburg, North-Western Russia. The first two species were originally classified as representatives of two distinct families, Burenellidae and Neopereziidae, respectively, basing upon morphological criteria of classical systematics of microsporidia. However, rDNA sequence analysis shows high level of homology (ca 95%) between these two haplotypes and their relatedness to the parasites of freshwater bryozoans, allocated to Clade V of molecular system, Class Aquasporidia (microsporidia of aquatic origin) sensu Vossbrinck, Debrunner-Vossbrinck 2005. The second three species are not so closely related (sequence similarity 77-83%) and all joined particular cluster uniting microsporidia of terrestrial origin infecting diverse hosts (ciliates, microcrustaceans, insects and human) nested within Clade IV, Class Terresporidia. These findings reveal polyphyletic origin of the microsporidia infecting one host, *Chironomus plumosus*, indicating possibility of evolutionary acquisition of microsporidian parasites by freshwater hosts from both freshwater and terrestrial habitats. The research is supported by RFBR no 10-04-00284 and a grant from RF President no MK-3419.2009.4.

Keywords: Microsporidia, chironomids, molecular phylogeny, rDNA

Contributed Paper Tuesday, 11:30 99-STU

Discovery of a novel microsporidian infecting commercial cultures of the Mediterranean cricket *Gryllus bimaculatus*

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We report the discovery of a novel microsporidian, infecting commercial pet food cultures of the Mediterranean cricket, *Gryllus bimaculatus* De Geer. Examination of calcofluor white-stained fat-body smears revealed the presence of single spores and groups of eight or sixteen spores enclosed within sporophorous vesicles. This feature is absent from *Paranosema grylli*, a characterised microsporidian pathogen of *G. bimaculatus*. Also unlike *P. grylli*, crickets infected with this novel species show no obvious pathology. PCR screening of eggs suggest vertical transmission of this parasite. These observations may indicate a more specialised co-evolution of host and parasite. Phylogenetic analysis of the 16S rDNA of the new species suggests that it is a sister group to the clade containing *Vavraia culicis* and *Trachipleistophora hominis*, found in *Culex* mosquitoes and immunodeficient human hosts, respectively. Both of these parasites also exhibit sporogony in sporophorous vesicles. Further characterisation of this species will be necessary to confirm its relationship to these significant microsporidian pathogens.

Keywords: Microsporidia, novel species, cricket host, *Gryllus bimaculatus*

Contributed Paper Tuesday, 11:45 100

Caught in the crossfire: Mismatch between morphological and molecular taxonomic data in classification of a novel microsporidian parasite of lobsters

Grant Stentiford[1]; **Kelly Bateman**[1]; **Hamish Small**[2]; **Jessica Moss**[2]; **Jeffrey Shields**[2]; **Kim Reece**[2]; **Ian Tuck**[1]
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Microsporidians are significant pathogens of aquatic crustaceans. Historically, their classification has been based upon the presence or absence of morphological features, but recent evidence derived from analysis of key gene sequences has demonstrated the potential for relatively high morphological plasticity in genetically similar species. This contradiction has led to significant confusion in the taxonomy of the phylum – a problem when attempting to classify newly discovered species for which both morphological and genetic data are available. Here we describe the first microsporidian parasite from Nephropid lobsters. Infected lobsters, displaying hyperpigmentation and lethargy, possessed skeletal and other muscles that were largely replaced by merogonic and sporogonic stages of the parasite. Transmission electron microscopy revealed diplokaryotic meronts, sporonts, sporoblasts and spore stages, all in direct contact with the host sarcoplasm. Analysis of the SSU rDNA gene sequence from the parasite suggested close affinity with *Thelohania butleri*, a morphologically dissimilar microsporidian from marine shrimp. Morphological features of the lobster parasite are more consistent with members of the family Nosematidae. The contradiction between morphological and molecular taxonomic data supported the erection of a new genus in which the lobster parasite is the type species (*Myospora metanephrops*). Furthermore, utilising the genetic-ecological classification system recently proposed by Vossbrinck and Debrunner-Vossbrinck (2005), we recommend the erection of a new family (Myosporidae) and a new order (Crustaceacida) to contain this genus, and other muscle infecting forms from marine crustaceans. The taxonomic framework presented could be further applied for re-classification of existing members of the Phylum Microsporidia for which appropriate morphological and molecular data are available and further, for novel microsporidian parasites discovered in a wide range of host phyla from marine, freshwater and terrestrial habitats.

Keywords: Crustacean, microsporidia, lobster

Contributed Paper Tuesday, 12:00 101

PCR identification, phylogeny analysis of Hsp70 gene of insect Microsporidia

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Nosema bombycis, as a typical pathogen of pebrine, always threatens the stability of silk industries. Are there being complicated cross infections between the pebrine pathogens and the wild insect microsporidians? Here microsporidia spores from silkworm moths, *Bombyx mori* and wild insects were collected from sericulture regions in China, respectively. Then we designed two sets of primer V1F/530R and KAI01N/KAI02N, and also developed the PCR-RFLP method for identifying the above spores. The result was showed that seven strains of microsporidia were closely related, and it was implied that they should be belong to the same species as *N. bombycis*. Furthermore, we use the methods of overlapping primers to amplify Hsp70 gene. Finally, a fragment of 1136 bp of Hsp70 gene was assembled with a set of primer of HspF/HspR for ten strains respectively. Distance matrix and phylogeny analysis showed that *N. bombycis* was closely related with the wild insect microsporidians. It was suggested that there was cross infection between pathogen of pebrine and wild insects; microsporidia were closely associated with their host. Hsp70 gene has genetic polymorphism; *N. bombycis* was a complex.

Keywords: Microsporidia, pebrine, phylogeny analysis, PCR

Contributed Paper Tuesday, 12:15 102

Intragenomic diversity of ribosomal DNA in *Nosema*

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In most species, ribosomal DNA (rDNA) units occur in tandem arrays and are homogenised by concerted

evolution. In contrast, the rDNA units of microsporidia are dispersed throughout the genome. I present evidence that the rDNA sequences of several *Nosema* species vary within isolates, probably due to variation between copies within the genome. This suggests that concerted evolution upon rDNA is weak in microsporidia and calls into question the use of rDNA sequences as markers to identify microsporidian strains.

Keywords: Ribosomal DNA diversity, Nosema recombination sex concerted evolution

12:30-13:45 LUNCH at KTU SAHIL

14:00-21:30 EXCURSION AND BBQ

18:00-21:30 BBQ. Buses returns to hotels

Wednesday July 14, 2010
Osman Turan Congress Centre

07:30 **Bus pick up at hotels**

08:00-09:30 **Posters Up:**
Bacteria, COST 862, Viruses and Fungi

Symposium Bacteria
Wednesday, 08:00-09:30
Hasan Turan

Insecticial Products from Bacterial Genome Sequencing
Organizer: Neil Crickmore

Symposium Wednesday, 08:00 103

The diversity of insecticidal toxins from bacteria
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In 1981 Schnepf and Whiteley reported the cloning of the first insecticidal toxin gene from *Bacillus thuringiensis*. In the 30 years since then the number of new toxin genes being discovered from Bt has continued to rise at a steady rate, and in addition insecticidal toxin genes have also been isolated from a variety of different species including other Bacilli, Clostridia and the nematode-inhabiting Xenorhabdus and Photorhabdus. In this era of rapid genome sequencing the potential exists to discover many more new toxins from these and other species, an exciting prospect but also one that has the potential to complicate the classification of these proteins. Examples are already appearing in public databases of toxin sequences being incorrectly annotated. This talk will start by summarising the range of toxins already classified and their grouping into particular classes. Some discussion will then follow about the relatedness between insecticidal toxins and mammalian-active bacterial toxins with particular reference to the parasporin class of Bt toxins. Finally we will contemplate the challenges ahead as a introduction to the subsequent talks which will outline the potential of identifying novel toxins through mass sequencing.

Keywords: Insecticidal toxin, *Bacillus thuringiensis*, Xenorhabdus, Photorhabdus

Symposium Wednesday, 08:30 104

Pesticidal gene discovery using *de novo* sequencing of bacterial genomes
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Pesticidal proteins from *Bacillus thuringiensis* (Bt) and related species have been identified primarily through empirical screening strategies, in which bacterial strains that show bio-activity towards target pests are characterized to identify active proteins. Expression of bacterial genes, however, is influenced by numerous environmental stimuli, including culture media, O_2 concentration, temperature, and the presence of metabolites. Consequently, laboratory growth conditions are unlikely to induce the expression of all bacterial pesticidal genes, which can prevent detection of protein activity during screening. Bt strains also frequently contain multiple pesticidal genes, some of which can have overlapping spectrums of activity. Active proteins from strains having multiple pesticidal proteins may be difficult to isolate using activity-based screening and fractionation strategies. By applying a combination of *de novo* sequencing and bioinformatics analysis, we have identified and cloned over 250 novel putative pesticidal proteins from Bt strains collected from a variety of environmental samples. These novel genes can be broadly categorized into several protein homology families: 3-domain delta endotoxins (Cry toxins), Mtx-like proteins, putative binary proteins, and Vip-like proteins. As part of a discovery pipeline designed to identify genes with potential application in agriculture, the genes encoding these putative pesticidal proteins have been cloned, and the proteins have been expressed and tested for activity against important agricultural pests. To date, we have identified new members of nearly every major group of the delta endotoxin family. Several novel proteins appear to represent new classes of proteins, and some appear to be fusions between known classes of pesticidal proteins. We are using these data to guide our discovery efforts, and to develop a refined model of pesticidal protein distribution across Bt strains.

Keywords: *Bacillus thuringiensis*, Cry toxins, *de novo* sequencing

Symposium Wednesday, 09:00 105

The Photorhabdus genome as a source of novel insecticidal products
Maria Sanchez-Contreras[1]; Andrea Dowling[2]; Paul Wilkinson[2]; Federico Dorati[3]; Robert Jackson[3]; Richard H. Ffrench-Constant[2]; Nick R. Waterfield[1]
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We have developed a novel approach, termed Rapid Virulence Annotation, or RVA (Waterfield et al., PNAS 2008), for the identification of bacterial virulence genes and toxins. RVA relies upon the screening of cosmid libraries from pathogenic bacteria against four model animals belonging to four diverse taxa. As a result this technique represents an excellent discovery tool for novel bioactive molecules including insecticides, antimicrobials and immune modulatory molecules. This approach proved to be an extremely valuable tool to identify functional virulence factors in the emerging human pathogen Photorhabdus asymbiotica ATCC43949. We have subsequently been using RVA to discover novel toxins in other in pathogens including the insect host restricted P.

luminescens TT01, the human pathogen Burkholderia pseudomallei, the fish pathogen Vibrio salmonicida and the plant pathogen Pseudomonas syringae. Of specific advantage is the ability of RVA to identify genes involved in the production of novel bioactive small molecules, or natural products. It is not possible to ascribe biological function to the products of non-ribosomal peptide or polyketide synthase gene clusters using bioinfomatic approaches. The genomes of Photorhabdus, and indeed many other bacterial strains, dedicate significant coding capacity to such secondary metabolite synthesis genes. RVA provides us with the means to access this resource and forms the base of a small molecule drug discovery pipeline.

Keywords: Photorhabdus, Bacterial virulence and toxins

Symposium Microsporidia

Wednesday, 8:00-10:30
Nihat Turan 1

Microsporidia and Other Pathogens in Arthropods from the Eastern Mediterranean Region.

Organizer: Andreas Linde

Symposium Wednesday, 08:00 106

Release of *Nosema lymantriae, Vairimorpha disparis* and *Entomophaga maimaiga* for classical and augmentative biological control of gypsy moth in Bulgaria and the United States

Daniela Pilarska[1]; Andreas Linde[2]; Plamen Pilarski[3]; Danail Takov[1]; Georgi Georgiev[4]; Leellen Solter[5]

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We conducted augmentative and inoculative releases of two microsporidian species, *Nosema lymantriae* and *Vairimorpha disparis* into gypsy moth populations in Bulgaria and the USA. Both species were originally isolated from gypsy moth populations in Bulgaria. The microsporidia were released in 2008 into two low density gypsy moth populations in Bulgaria and two rising populations in northern Illinois, USA. We monitored the releases in 2008 and 2009. In 2008, *N. lymantriae* and *V. disparis* were recovered from hosts in both sites in Bulgaria, only *N. lymantriae* was detected in 2009. No infected larvae were collected in Illinois sites in 2008. In Illinois, the introduced fungal pathogen, *Entomophaga maimaiga*, was epizootic in the gypsy moth study sites. It is not known if *E. maimaiga* competes with microsporidia in host populations. *E. maimaiga* was inoculatively introduced as a classical biological control agent into six different gypsy moth populations in Bulgaria during the period 1996-2009. Monitoring studies showed that the fungus had successfully established in 9 sites by 2009, but it is not yet present in the microsporidia release sites. The presence of a new biological control agent, *E. maimaiga*, in Bulgaria has potential to allow the reduction of the use of pesticides in the native gypsy moth, while establishment of microsporidia in North America would add to the natural enemy complex where gypsy moth is an introduced pest.

Acknowledgements: We thank Deutsche Forschungsgemeinschaft, the National Science Fund of Bulgaria grant DO-02-282/2008, USDA Forest Service no. 06-JV-11242300-045 and Agricultural Experiment Station Project no. ILLU-65-0344, USDA FS Cooperative Agreement no. AG 01CA-11242343-107

Keywords: *Nosema lymantriae*, *Vairimorpha disparis*, *Entomophaga maimaiga*, release

Symposium Wednesday, 08:25 107

Pathogens of forest pest insects in Georgia

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Georgian forests take a distinguished place on the Eurasian continent because of their biodiversity and high number of endemic species. Periodically outbreaks of insect pests occur, often above the economic damage thresholds.

One of the most aggressive pest insects in Georgia is the spruce bark beetle *Ips typographus* L, causing considerable damage to *Picea orientalis* L. Another serious pest is the defoliator *Lymantria dispar* L (gypsy moth), which is considered to be a chronic pest. In the Dusheti district the pest occurred in 60% of the area and defoliated 20% of endemic oak trees.

Several pathogens of *I. typographus* and *L. dispar* were revealed from different sites in Georgia. A brief characterization of the pathogens will be presented.

Gregarina typographi (Protozoa, Eugregarinida) was observed in the midgut lumen of bark beetles from every investigated site. The prevalence of this pathogen varied at different altitudes and different sampling plots.

The microsporidium *Chytridiopsis typographi* was also found in the midgut epithelium of *I. typographus*. It´s prevalence was relatively low.

A high natural mortality of gypsy moth was registered at the outbreaks sites in Georgia. From dead larvae we isolated a NPV and a fungus belonging to the entomophthorales. A rapid decline in the number of pest occurred in the following year in places where high mortality was observed in the previous year. However, population density of *L. dispar* remained high in places where mortality had been quite low (between 5-7%).

Results on the field and laboratory studies will be presented.

Keywords: *Ips typographus* L., *Lymantria dispar* L., pathogens, forest

Symposium Wednesday, 08:50 108

An overview on entomopathogenic protists from Turkey

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Investigations on entomopathogenic protists in Turkey are quite limited. In this presentation, a brief review of the current status of research on entomopathogenic protists from Turkey will be given. So far, microsporidia have been recorded from three insect groups: In the first group are agriculturally important pests, here the members of the family Chrysomelidae. The microsporidian infections in these insects are regarded as a possible options for use in biological control of the beetles. The second group in which microsporidia were found are predatory insects, which are being used in biological control. In the third group are beneficial insects with an economic and ecological importance, including the honey bee *Apis mellifera* and the silkworm *Bombyx mori*. Infections in these latter groups are undesirable because of their economic importance. Until today, seven microsporidia species were recorded: Five species belong to the genus *Nosema: N. apis, N. phyllotretae* Weiser 1961, *N. leptinotarsae* Lipa 1968, *N. chaetocnemae* Yaman et Radek 2003, *N. tokati* Yaman et al. 2008, and *N. rhaphidae* Yaman et al. 2009. One species belongs to the genus *Unikaryon: U. phyllotretae* Yaman et al. 2010. Another microsporidium has not been identified on the species level yet. Four of these seven species were recorded from their hosts for the first time and described as new species. Besides microsporidia, members of the genus Gregarina were found in bark beetles (Ips typographus and Ips sexdentatus) and some chrysomelid species. Effects of gregarines on the host insect were also investigated.

Keywords: Microsporidia, Turkey, gregarines, review

Symposium Wednesday, 09:15 109

Pathogen infections of the bark beetle *Dendroctonus micans* and its predator *Rhizophagus grandis* from Turkey

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The great spruce bark beetle *Dendroctonus micans* (Curculionidae, Scolytinae) causes serious economic losses in Turkey, despite the large efforts to mechanically or chemically control the beetle. The introduction of a specific, mass-reared predator of *D. micans*, the beetle *Rhizophagus grandis* (Rhizophagidae), is an important factor in confining the *D. micans* population. Another possibility to regulate the density of the bark beetle would be the detection and use of natural pathogens of this pest. Recently, we discovered several infections of *D. micans*. For the first time, we found the parasitic green alga *Helicosporidium* sp. to infect a bark beetle. Its typical spores (diameter 4.9 µm) contain three ovoid cells and one helical, filamentous cell. Furthermore, infections with the yeast *Metchnikowia* sp., the neogregarine *Mattesia* sp. and a nematode were recorded for the first time. In fresh smears, the elongated asci of *Metschnikowia* measure 18.1 x 2.1 µm. The typical navicular spores (10.9 x 6.1 µm) of *Mattesia* have a plug at each cell pole and are formed in pairs within a gamontocyst. Since *R. grandis* feeds on larvae of *D. micans*, a transmission of pathogens to the predator might be possible and could reduce the efficiency of controlling its prey. In fact, we found a *Helicosporidium* and a *Mattesia* infection in *R. grandis*.

Keywords: Helicosporidium, Mattesia, Metschnikowia, bark beetle, Dendroctonus, Rhizophagus

Symposium Wednesday, 09:40 110

Entomopathogenic fungi as potential microbial control agents against hazelnut and forest pests in the Black Sea Region of Turkey

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The Black Sea Region of Turkey is popular with 70% world hazelnut production and 32% Turkey's total forest. Several harmful insects cause serious economic losses on both hazelnut production and forest lands each year since they cannot be effectively controlled by various methods. Microbial control of pests with entomopathogenic fungi is an attractive alternative and a most appropriate approach in the Eastern Black Sea Region due to climatic conditions of the region. Several promising fungal studies have been done to find out some microbial control agents against the important hazelnut and forest pests in Turkey. 62 fungal samples from soil and 18 fungal samples from insect pests have been isolated from the Black Sea Region of Turkey, identified and tested for insecticidal activities. This presentation provides an overview of fungal insect pathogens isolated from soils and some forest pests in this region and their insecticidal activities against a number of pest species.

Keywords: Black Sea Region, Entomopathogenic fungi, Hazelnut, Forest

Symposium Wednesday, 10:05 111

Release of *Nosema lymantriae, Vairimorpha disparis* and *Entomophaga maimaiga* for classical and augmentative biological control of gypsy moth in Bulgaria and the United States

Daniela Pilarska[1], Andreas Linde[2], Plamen Pilarski[3], Danail Takov[1], Georgi Georgiev[4], Leellen Solter[5]
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The gypsy moth *L. dispar* periodically causes severe damage in forests in several Central and Eastern European countries, and in the USA. In Bulgaria, oak stands of different ages are infested with gypsy moth populations in varying densities over long periods of time (Georgiev et al. 2007). Currently, the bacterial pathogen *Bacillus thuringiensis* var. *kurstaki* (*Btk*) and broad spectrum insecticides are used to control outbreaking gypsy moth populations. Because of the cost of spraying and environmental concerns it may be more judicious to rely on the suppressive effects of natural enemies that cycle in the host population. Host specific gypsy moth microsporidia and the entomopathogenic fungus *Entomophaga maimaiga* are examples of biological agents that have been reported to have long term persistence (Pilarska 1998; Hajek et al. 2004) and reduce high density populations (Sidor, 1979; Zelinskaya 1980; Sidor et al. 1983; Solter and Hajek 2008).

Results of laboratory and field studies showed that the that the gypsy moth pathogenic microsporidia *Nosema lymantriae* and *Vairimorpha lymantriae* have a very narrow host range (Solter et al. 2000, 2005, 2010). Both species were originally isolated from gypsy moth populations in Bulgaria. In order to evaluate the success of establishment and persistence of *N. lymantriae* and *V. disparis*, we conducted augmentative and inoculative releases of both microsporidia into gypsy moth populations in Bulgaria and the USA.

Infected third-instar *V. disparis* larvae were released in 2008 into low density gypsy moth populations in the Northwest of Bulgaria (Opletnya), and *N. lymantriae*-infected larvae were released in a site in the Southwest (Karlanovo)., Both microsporidian species were collected in the experimental gypsy moth populations 15 days after release. Of the collected larvae in Karlanovo, 54.8% were infected with *N. lymantriae*. Similarly, in Opletnya site 57.1% were infected with *V. disparis*.

In 2009, 8.1% of larvae collected in Karlanovo were infected with *N. lymantriae*; none of the gypsy moth larvae recovered from Opletnya site were infected with microsporidia. No non-target larvae from the release sites we collected in 2008 and 2009 were infected.

Using the same methods of host rearing, infection and release, *N. lymantriae* and *V. disparis* were released into two rising gypsy moth populations in northern Illinois, USA in 2008. *V. disparis* was released in Chain 'O Lakes State Park and *N. lymantriae* was released in Volo Bog State Natural Area, both in McHenry Co., Illinois. Monitoring in late season 2008 revealed an intensive epizootic of *E. maimaiga* and neither microsporidium was recovered; nor were infected larvae recovered in 2009. The *E. maimaiga* epizootic continued in 2009. The microsporidia were released again in the same sites in 2010.

Entomophaga maimaiga was imported from the USA and introduced via *L. dispar* cadavers containing resting spores of the fungus in seven different *L. dispar* populations in the region of State Forestries Svoge (Northwest Bulgaria, 1996 and 2001), Karlovo (Central Bulgaria, 1999), Assenovgrad (South Central Bulgaria, 2001), Stryama (South Central Bulgaria, 2005), Nova Zagora (Central Bulgaria, 2008), Popovo (Northeast Bulgaria, 2009) and Gorna Oriahovitsa (Northeast Bulgaria, 2009).

In 2005, *E. maimaiga* epizootics occurred at four different sites in Bulgaria (SF Haskovo, Kirkovo, Botevgrad and Govezhda) located 30-70 km from the introduction sites in 1999 and 2000 (Georgiev et al., 2007; Pilarska et al., 2007). Monitoring studies showed that *E. maimaiga* was recovered in two more localities in 2009 – SF Ravna gora (Northeast of Bulgaria, 160 km from Nova Zagora release site) and SF Zvezdets (Southeast of Bulgaria, 120 km from Nova Zagora release site). The fungus had successfully established in 9 sites by 1999-2009, but it is not yet present in the microsporidia release sites.

The presence of a new biological control agent, *E. maimaiga*, in Bulgaria has potential to allow the reduction of the use of pesticides for control of the native gypsy moth, while establishment of microsporidia in North America would add to the natural enemy complex where gypsy moth is an introduced pest. Future studies are warranted on competition between the microsporidia and *E. maimaiga*.

Acknowledgements: We thank Deutsche Forschungsgemeinschaft, the National Science Fund of Bulgaria grant DO-02-282/2008, USDA Forest Service no. 06-JV-11242300-045 and Agricultural Experiment Station Project no. ILLU-65-0344, USDA FS Cooperative Agreement no. AG 01CA-11242343-107.

Contributed papers

Wednesday, 8:00-10:00
Fahri Kuran

VIRUSES 4

Chairs: Bob Harrison and Nor Chejanovsky

Contributed Paper **Wednesday, 08:00 112**

RNase III expressed by ascoviruses and its potential roles in host-virus interactions

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Ascoviruses cause lethal infections in their host larvae diagnosed by milky-white colouration of hemolymph due to accumulation of virus-containing vesicles. Recently, genome sequences of four ascoviruses have become available providing a springboard to analyze their evolutionary relationships with other invertebrate viruses and the role of expressed genes in host-virus interactions. All the four sequenced ascoviruses encode a gene with significant similarities to RNase III. There are only few DNA viruses known to encode RNase III. In this study, we investigated the pattern of expression of *Heliothis virescens ascovirus* (HvAV3e) RNase III gene (*orf27*) in the host, confirmed its ribonucleic acid activity and explored its potential roles in host-pathogen interactions. The gene is expressed early in infection but autoregulates its expression by endonuclease activity. The expressed recombinant protein exhibited RNase activity against double stranded RNA. RNA interference studies indicated that the gene is essential for virus DNA replication and infection. Moreover, we found that orf27 is involved in suppression of gene silencing and potentially in microRNA biogenesis.

Keywords: Ascovirus, RNaseIII, gene silencing

Contributed Paper **Wednesday, 08:15**
113-STU

AcMNPV IE-1 plays an important role in the development of virogenic stroma in the baculovirus life cycle

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In the early stage of the baculovirus *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) infection of *Spodoptera frugiperda* cells, host heterochromatin is generally redistributed and becomes marginated along the inner nuclear membrane; meanwhile, the viral replication center, called virogenic stroma (VS), forms progressively. However, the molecular basis for such changes in nuclear architecture and factors governing the assembly of VS are unknown. In this study, an AcMNPV immediate early gene *ie-1* knockout bacmid was generated through homologous recombination in *E. coli*. *Polyhedrin* and *gfp* were inserted into the *polh* locus of the bacmid through transposition by using Bac-to-Bac system to generate $vAc^{ie\text{-}1KO\text{-}PH\text{-}GFP}$. Immunoelectron microscopy showed that the changes of heterochromatin and the formation of VS were not observed in cells transfected with $vAc^{ie\text{-}1KO\text{-}PH\text{-}GFP}$. Interestingly, transient expression of *ie-1* induced heterochromatin rearrangement in Sf9 cells. These results indicate that IE-1 plays an important role in the development of VS in the baculovirus life cycle.

Keywords: AcMNPV, ie-1, virogenic stroma

Contributed Paper **Wednesday, 08:30 114**

Strong activation of viral genes in mammalian cells by baculovirus IE2 depends on a novel viral nuclear structure

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The BacMam system has been developed as a safe, fast and economical alternative for foreign protein production in mammalian cells. BacMam, a baculovirus which carries a foreign gene under a mammalian promoter (mostly the CMV*ie* promoter), can now be effectively transduced into a wide range of mammalian cells. To maximize BacMam's potential, we discovered a baculovirus immediate-early (IE) protein, IE2, which could dramatically up-regulate the expression level of the target gene. The mechanism behind IE2 activation of target genes was closely linked to a unique nuclear structure, the IE2 nuclear body (NB). It resembled a dynamic spherical ball with IE2 proteins forming the surface, and filled by monomeric G-actin within. As well as G-actin, the presence of large active Pol II foci and nascent viral transcripts indicated that it was an active viral transcription center. Interestingly, IE2-expressing baculovirus transduced mammalian cell nuclei had less PML NBs than wild-type virus transduced cells, given rise to the possibility that IE2, an ubiquitin ligase similar to ICP0 of herpes simplex virus-1, can also disrupt PML NBs. Although baculovirus and herpes viruses are evolutionally distant, this rare coincidence showed that compartmentalizing host nuclei by IE viral factors is a well established viral strategy. These compartments serve multiple purposes that often include harvesting host transcription machinery for viral gene transcription and disrupt host anti-viral defense complexes (like PML NBs). Further understanding of host-virus interactions at these viral nuclear structure sites will greatly enhance the use of baculovirus in the field of mammalian protein expression.

Keywords: BacMam, IE2, Mammalian protein expression

Contributed Paper **Wednesday, 08:45 115**

Identification and mutagenesis of cysteine residues which play critical roles in the formation of intersubunit disulfide bridge of mature d F Protein of *Helicoverpa armigera* single nucleocapsid nucleopolyhedrovirus

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The mature F protein of baculovirus budded virus (BV) consists of two subunits, the N-terminal subunit F_2 and C-terminal membrane-anchored subunit F_1. The two subunits are linked by a disulfide bond. Eleven cysteine residues are found highly conserved in the baculovirus F proteins and F-like proteins. To investigate cysteine residues which play significant role in the formation of the intersubunit disulfide bond and virus infectivity, mutagenesis analyses were performed on cysteine residues of the F protein of HearNPV. The function of mutated HaFs were detected by rescuing the infectivity of *f*-null bacmid of HearNPV (Habac) and the formation of intersubunit disulfide bond was detected by expressing the mutated HaFs in the bacmid of AcMNPV (Acbac). The results demonstrated that: 1) the intersubunit disulfide bridge in functional mature F protein is formed between C108, the only cysteine residue in F_2 subunit, and C241, the conserved cysteine residue in the N-terminal of F_1 subunit, 2) C232, as a relatively conserved cysteine resides, could form intersubunit disulfide bond with C108 when C241 was mutated, but the mutated protein could not rescue the infectivity of *f*-null Habac. No intersubunit bridge was observed in the dual mutations of C232 and C241, and the mutant impaired the ability of HaF in rescue viral infectivity dramatically, 3) The mutation at C403 had no obvious effect with intersubunit disulfide bond formation but interfered viral infectivity. This suggests that C403 could be a potential cysteine residue forming intrasubunit disulfide bond.

Keywords: Baculovirus, HaF, cysteine residue, disulfide bond

Contributed Paper Wednesday, 09:00 116

AcMNPV ME53 co-localizes with GP64 at viral budding sites

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We demonstrated that deletion of *me53* from the AcMNPV genome results in a significant (1000- 10 000 fold) reduction in budded virus production from Δme53 bacmid transfected *Sf*-21 cells. In order to elucidate the function of ME53, a Δme53 repair virus expressing a ME53:GFP fusion under control of the native *me53* early/late promoter was generated. The repair virus, AcΔme53RepME53:GFP, produced an expected 80 kDa protein detectable with anti-GFP antibodies by Western blot analysis and produced levels of budded virus similar to those from the wildtype virus indicating that the ME53 activity of the fusion protein was not compromised. Confocal fluorescent imaging of live cells at early times post infection (6-12 hours) revealed a mainly cytoplasmic distribution of ME53:GFP, while at later times, 18-36 hours post-infection, the distribution was predominantly nuclear and discrete foci formed at the plasma membrane. Moreover, immunofluorescent confocal imaging with anti-Gp64 (AcV1) antibody revealed strong co-localization of GP64 and ME53:GFP at these foci. Quantitative analysis of co-localization revealed an average Pearson's coefficient of 0.768 and an average overlap coefficient of 0.976 which supports the dye overlay data. Further, immuno-gold transmission electron microscopy demonstrated that ME53 concentrates in foci just below the plasma membrane. These discrete areas at the plasma membrane where ME53:GFP and GP64 co-localize might represent viral budding sites in which ME53 either recruits nucleoccapsids to these sites or may be more directly involved in the budding process.

Keywords: *me53*, gp64, baculovirus budding, confocal microscopy

Contributed Paper Wednesday, 09:15 117

***Autographa californica* multiple nucleopolyhedrovirus *ac76* is involved in intranuclear microvesicle formation**

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In this study, we characterized *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) orf76 (*ac76*), which is a highly conserved gene of unknown function in lepidopteran baculoviruses. Transcriptional analysis of *ac76* revealed that transcription of multiple overlapping multicistronic transcripts initiates from a canonical TAAG late-transcription start motif but terminates at different 3′ ends at 24 h postinfection in AcMNPV-infected Sf9 cells. To investigate the role of *ac76 in the baculovirus life cycle, an ac76*-knockout virus was constructed using an AcMNPV bacmid system. Microscopy, titration assays and Western blot analysis demonstrated that the resulting *ac76*-knockout virus was unable to produce budded viruses. Quantitative real-time PCR analysis demonstrated that *ac76* deletion did not affect viral DNA synthesis. Electron microscopy showed that virus-induced intranuclear microvesicles as well as occlusion-derived virions were never observed in cells transfected with the *ac76*-knockout virus. Confocal microscopy analysis revealed that Ac76 was predominantly localized to the ring zone of nuclei during the late phase of infection. This suggests that *ac76* plays a role in intranuclear microvesicle formation.

Keywords: AcMNPV, ac76, microvesicle formation

Contributed Paper Wednesday, 09:30 118

A non-coding RNA of *Hz*NV-1 virus establishes latent viral infection through microRNA

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Mechanism controlling latent viral infection is a subject of intense studies. In this report, *Heliothis zea* nudivirus (*Hz*NV-1), an insect virus able to establish both productive and latent viral infections in insect cells, was used as a model system for studying such phenomenon. During productive infection, this virus generates more than 100 transcripts. One of these, *hhi1*, is a major early transcript that can induce strong apoptosis, and is responsible for the establishment of productive viral infection. Interestingly, during latent viral infection, a non-coding RNA, persistency-associated transcript 1 (PAT1), is the only viral transcript expressed. We found that PAT1 can efficiently block *hhi1*-induced apoptosis. Further studies showed that PAT1 encodes two distinct microRNAs, miR-2959-5p and miR-246-5p, which target and degrade *hhi1* transcript for the establishment of latent viral infection. These results provide a novel mechanism for the functioning of a non-coding RNA, and provide a unique example of miRNA releasing from a non-coding RNA to promote viral latency.

Keywords: *Heliothis zea* nudivirus (HzNV-1), microRNA

Contributed papers
Wednesday, 8:00-10:00
Nihat Turan 2

NEMATODES 1

Chairs: Ed Lewis and Selçuk Hazir

Contributed Paper Wednesday, 08:00 119

Genes that are involved in the recovery process in the entomopathogenic nematode *Heterorhabditid bacteriophora*

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Recovery in nematodes defines as: exit from developmental arrest and resuming growth and development. In entomopathogenic nematodes this process occurs when the infective juvenile (IJ) enters the insect host, as a response to insect hemolymph. The recovery process is the first outcome of the host-parasitic interaction and is also a commercially very important process. This study was aimed at identifying genes that are putatively involved in this process in *Heterorhabditid bacteriophora*. For this purpose, a large scale bioassay for recovery was established and two subtractions libraries of recovered IJs subtracted by arrested IJs were constructed. Six hundreds expressed sequence tags (ESTs) were sequenced and annotated, resulting in 300 useful ESTs that were compared to *C. elegans* Wormbase and categorized into functional categories according to gene ontology. Of these, twenty three genes were further analyzed. Their expression in the recovery process was determined by quantitative (q) RT-PCR. The expression pattern supported the results obtained from the subtraction libraries. Further analysis of these genes was done by RNAi-based functional analysis in *H. bacteriophora*. Silencing twenty three genes by dsRNAi result in different phenotypes. Bioassay for recovery of IJs harboring different silenced gene was preformed. We found 8 genes that when silenced reduced IJs recovery dramatically compared to recovery in WT IJs of *H. bacteriophora*. Thus these genes are critical in the recovery process. The relation of six of these genes to recovery requires further study. However, the other two genes are connected to insulin/IGFI pathway which is known to regulate dauer formation in *C. elegans*.

Keywords: *Heterorhabditid bacteriophora*, RNAi-based functional analysis

Contributed Paper Wednesday, 08:15 120

Exploring the molecular basis of 'Recovery' in infective juveniles of the entomopathogenic nematode *Heterorhabditis bacteriophora* TTO1

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Among entomopathogenic Steinernematid and Heterorhabditid nematodes, 'Recovery' is defined as the process in which the infective juvenile resumes development, following invasion into the hemocel of the insect host, to the reproductive stage. Characterizing the molecular basis of the recovery process in *H. bacteriophora*, was done by identifying genes that are putatively involved. For this purpose, a large scale bioassay for recovery was established and two subtractions libraries of recovered IJs subtracted by arrested IJs were constructed. Six hundreds expressed sequence tags (ESTs) were sequenced and annotated resulting in 300 useful ESTs that were compared to *C. elegans* Wormbase and categorized into functional categories according to gene ontology. Of these, twenty three genes were chosen for further analysis. These genes were examined for their expression in the recovery process by quantitative (q) RT-PCR. The results of the RT-qPCR supported the results obtained from the subtraction libraries. Further analysis of these genes was done by RNAi-based functional analysis in *H. bacteriophora*. Eight genes displayed significant reduction in recovery as compared to control treatment as well as low expression and measured by RT-qPCR. The possible role of selected genes will be presented.

Keywords: EPN, recovery, RNAi

Contributed Paper Wednesday, 08:30 121

***Steinernema* nematodes and their bacterial endosymbionts: A multigene approach to inferring their evolutionary histories**

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Steinernema nematodes (Steinernematidae) and their mutualistic bacteria *Xenorhabdus* spp. (γ-Proteobacteria) are an emergent model of terrestrial animal-microbe symbiosis. Interest in this association initially arose out of their potential as biocontrol agents against insect pests, but despite advances in their field application and the growing popularity of this model system, relatively little has been published to uncover the evolutionary facets of this beneficial partnership. This study adds to the body of knowledge regarding nematode-bacteria symbiosis by proposing a possible scenario for historical association in the form of a cophylogenetic hypothesis. Topological and likelihood based testing methods were employed to reconstruct a history of association between 29 host-symbiont pairs, and to gauge the level of similarity between their inferred phylogenetic patterns.

Keywords: Co-phylogeny, Steinernema, Xenorhabdus, host switching, horizontal transfer

Contributed Paper Wednesday, 08:45 122

Diversity, distribution, and phylogenetic analysis as inferred from ribosomal DNA sequences of the ITS1-5.8S-ITS2 region of entomopathogenic nematodes in Pakistan

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Entomopathogenic nematode (EPNs) is a new type of bio-pesticide which has great potential for conrolling many important agricultural pests. Entomopathogenic nematode (EPNs) distribution in Pakistan (Sindh, Punjab, Balochistan and NWFP) has been studied on the basis of four different climatic regions: the Coastal area the Upper Indus plain and lower Indus valley, the North and North Western Mountainous area, and the Plateau of Balochistan and their desert. Throughout Pakistan six indigenous species of Steinernematid *Steinernema pakistanense* (Ham 10 strain); *S. asiaticum* (211 strain); *S. siamkayai* (157 strain); *S.*

abbasi (507 strain); *S. feltiae* (A05 strains); *S. carpocapsae* (T51 strain); and two species of heterorhabdus *H. indica* HAM-64 strain); *H. bacteriophora* (1743 strain); were isolated. Steinernematid were more ubiquitous then the Heterorhabditis. The ITS1-5.8S-ITS2 regions of rDNA, of 9 strains from Pakistan were PCR amplified and cloned. Sequences of these fragments were analyzed and phylogenetic trees were constructed with *Caenorhabditis elegans* as out group taxon. Phylogenetic relationships among these strains were estimated. One of these strains MM7 belongs to *Heterorhabditis* and phylogenetic analysis showed that it belongs to *H. indica*. The other 8 strains were *Steinernema*. Phylogenetic analysis showed that strains M24, M33, G6, G98 and K8 belongs to *S. siamkayai*, and strain 105, 109 are *S. pakistanense*, while strain T51 close to *S. carpocapsae*. The diversity of EPN species in Pakistan were studies on climatic basis, for getting the successful results to be used in biocontrol and IPM program throughout the country.

Keywords: Diversity, entomopathogenic nematode, *Steinernema heterorhabdus*, phylogenetic relationships

Contributed Paper Wednesday, 09:00 123

An outlook on Italian EPN biodiversity

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A mapping of EPN distribution in Italy, still in course, showed a high number of EPN species if compared with European and Mediterranean Countries. In the last 25 years, up to 1,500 soil samples were collected in almost all Italian regions. The EPN were isolated from agricultural fields, woodlands, sea beaches, coastal zones, uncultivated lands, river and lake borders. A total of 122 strains were collected belonging to 10 species: 38 strains of *Heterorhabditis bacteriophora*, 50 *Steinernema feltiae*, 11 *S. affine*, 4 *S. kraussei*, 8 *S. apuliae*, 3 *S. ichnusae*, 3 *S. carpocapsae*, 3 *Steinernema* strain S.sp.MY7 of *S. intermedium* group, and 2 different *Steinernema* sp. of *S. arenarium* group (ItS-ESC1 and ItS-C31 strains, currently under description). *S. kraussei* was collected only around Etna Vulcan in Sicily and 4 new species *S. apuliae*, *S. ichnusae*, *Steinernema* spp. ItS-ESC1 and ItS-C31 were identified. Interesting data are related also to the genetic and morphological variability of the different populations inside each species (i.e. the 50 *S. feltiae* strains collected can be divided in 2 genetic groups and at least 11 different morphological groups). Concerning the symbiotic bacteria the study is already started and still in progress. The high EPN biodiversity found in Italy could be related to the unique geographic shape of this country, longer than wide and to the segregation of the island fauna.

Keywords: Steinernematidae, entomopathogenic nematode, new species, Heterorhabditidae, distribution, Italy

Contributed Paper Wednesday, 09:15 124

Novelty in distribution of entomopathogenic nematodes

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Entomopathogenic nematodes (EPNs) are widespread, reported from all continents (excluding Antarktis).
The surface of the globe may be divided into five great divisions with respect to latitude and average year temperature – two frigid zones (arctic and antarctic), two temperate zones (southern and northern), and one torrid zone (tropics). Such divisions support fauna and flora typical of each zone.
Most EPN species have been recovered from temperate environments, however there is a recent effort for surveys in tropical and subtropical regions that brought some unbelievable links in species distribution. Both temperate and torrid zones are rich in suitable insect hosts for EPNs while frigid zones, due to short vegetation periods are poor in an insect biodiversity, and represent marginal areas for the EPN occurrence.
Several EPN species have a world wide distribution in the temperate and torrid zones. For example, *Steinernema feltiae*, *S. carpocapsae* and *Heterorhabditis bacteriophora* are examples of ones that overlap several zoogeographical regions. This situation seems to be unique in the animal world when compared for example with butterflies. Recently performed surveys in Africa and South America reported some incredible, difficultly explicable, distribution of *S. weiseri* (links between Czech Republic and Kenya) and *S.websteri* (links between temperate China and Colombian tropicals). However, the EPN fauna of all continents is not completely known. *S. affine* is an example of how deficient is our knowledge concerning the EPN distribution. This species, previously reported strictly for Europe, was found common in the southwest coast of British Columbia where the species could be introduced by settlers. Heterorhabditids are found less frequently than steinernematids and they seem to be better adapted to warmer, coastal, lowland areas, and habitats with light, sandy soil. Steinernematids inhabit a broader spectrum of ecosystems and habitats at various altitudes and they may pursue insect hosts in marginal areas of their distribution, such as 3500 m above sea level in the Himalaya and Tibetan Mts. The group of species that are phylogenetically close to *S. glaseri* are characterized by having the longest infective juveniles and relatively similar morphology. Their distribution seems to be patchy, but covering all continets. Some species of this group likely occur endemically due to space island isolation, for example,

S. cubanum in Cuba and *S. puertoricense* in Puerto Rico.
Number of described species have raised significantly in last years. In 2002 in the chapter "Biogeography" by William Hominick (Entomopathogenic Nematology edited by R. Gaugler) 26 steinernematids and 9 heterorhabditids were mentioned. Five years later in the book "Entomopathogenic nematodes: systematics, phylogeny and bacterial symbionts" edited by K.B.Nguyen and D. Hunt, 55 steinernematids and 11 heterorhabditids were characterized. Presently at least 60 and 12 species, respectively, are classed with these two families. Beside an elementary morphology the important role in the EPN recovery represents a DNA analysis.
and distributed in most terrestrial ecosystems and habitats. In the field, they parasitize mainly those insect stages that inhabit a soil environment or some humid, cryptic microhabitats. Numerous publications show data concerning the distribution of EPNs in cold, mild, and warm environments.

Keywords: EPN, Steinernema, Heterorhabditis, zoogeographical regions, terrestrial ecosystems, survey

Contributed Paper **Wednesday, 09:30**
125-STU

Development of a cultivated system with insect cells for an entomopathogenic nematode *Steinernema carpocapsae*, and analysis of the recovery

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A new method for culturing an entomopathogenic nematode *Steinernema carpocapsae*, in cultured insect cells under axenic conditions was devised (the cell-cultured system). When *Steinernema* eggs were put into the insect medium cultured the established cell line Sf9, they grew, moulted, developed to adults and produced eggs. Their life cycle took about 6 days and successive subcultures were possible. Each developmental event could be observed easily through the transparent culture vial under a microscope. *S. carpocapsae* show 'recovery' from the dauer form as infective juveniles (IJ) up to fourth-stage juveniles when host invasion. This recovery also occurs within an insect cell-cultured system. Here we addressed the factor(s) that induce recovery. Insect cell lines such as silkworm (*Bombyx mori*)-derived BmN cells and fruit fly (*Drosophila melanogaster*)-derived S2 cells also have activities for the recovery. The recovery was induced by insect cell secretes. By contrast, mammalian cells (NIH/3T3 and HeLa) had no effect on nematode recovery. These results suggest that *S. carpocapsae* might not recover within mammals. The observation of non-recovery of *S. carpocapsae* within mammalian cells provides additional evidence for the safety of this nematode as a biological control agents.

Keywords: Recovery, IJ, insect cell

Contributed Paper **Wednesday, 09:45 126**

Diversity and biogeographic distribution of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in Lebanon

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Entomopathogenic nematodes (EPNs) are remarkably resourceful in being useful biological control agents against many soil insect pests. They are ubiquitous, having been isolated from every inhabited continent (except Antartica) from a wide range of ecologically diverse soil habitats including cultivated fields, forests, grasslands, deserts, and even ocean beaches. Lebanon is among the few countries for which no data on EPNs and their symbiotic bacteria is available. We analyzed for the first time the diversity and the distribution of entomopathogenic nematodes in the families Heterorhabditidae and Steinernematidae throughout an extensive biogeographical survey in Lebanon during 2008 and 2009. Samples were collected in the coastal strip and in 9 vegetation types extending from sea level to 3088m above sea level. Sampling framework consisted of 570 (19x30) samples extracted from 19 sites in the different levels. Within each vegetation type, wooded and herbaceous ecosystems were considered for sampling purposes. 6 samples among 570 were positive representing three positives sites among 19 (16%). Two EPN species *Heterorhabditis bacteriophora* and *Steinernema feltiae* were recovered.

Keywords. Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae), symbiotic bacteria (*Xenorhabdus* et *Photorhabdus*), biodiversity, natural ecosystems, Lebanon.

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

Symposium Viruses-2
Wednesday, 10:30-12:30
Hasan
Turan

Arthropod Transmitted Viral Diseases

Organizers: Hu Zhihong (Rose) and Gorben Pijlman

Symposium **Wednesday, 10:30 127**

CCHFV-host interaction and the current situation in Turkey

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Ticks are among most successful vectors together with mosquitoes. About 10% of 899 known tick species are responsible of transmission of about 200 agents. Among 222 tick species recorded to bite humans, only 33 are known as frequent feeders on humans, and 28 of them are directly associated with transmission of disease agents to humans.
CCHF is the most widespread tick-borne viral disease of humans. It has been recorded from more than 30 countries. Although humans acquire the infection mainly via tick bites, crushing of infected ticks or

contact with viraemic animals blood or tissues are also source of infection. Nosocomial infections due to contact with infected patients are also seen.
Although CCHFv has been isolated from about 30 tick species, the vector competence has been demonstrated only for limited number of tick species, among which *Hyalomma* species are strictly associated with the global distribution of the disease. The tick species associated with the current CCHF epidemic in Turkey is *H. marginatum*. The same species is known to be involved in CCHF outbreaks in Balkans, Crimea and Southern Federal Districts of the Russian Federation. *Hyalomma anatolicum* in Iran, Pakistan, Turkmenistan and Tadjikistan; *Hyalomma asiaticum* in Central Asia and China, *Hyalomma rufipes* in Africa are suggested as the main vectors of CCHF. It is important to mention that in CCHF cases out of *H. marginatum*'s areal are highly correlated with animal butchering together with tick bites.
CCHF epidemics in Balkans, Crimea, Southern Federal Districts of Russia have been always associated with ecological changes leading to increase of wild animals and *H. marginatum* population.
In Turkey first cases were diagnosed in 2002 and until the end of 2009 a total of 4448 cases were reported from 2200 rural settlements with overall mortality of 4.9%. In Turkey it has been shown that *H. marginatum* is the dominant species in the CCHF areas. It has been also demonstrated that *H. marginatum* is transmitting the virus both transovarrialy and transstadially and 16.43% of host seeking *H. marginatum* ticks were CCHFv infected (unpublished results). Disease risk is strongly associated with presence of H. marginatum and habitat fragmentation.

Keywords: Crimean-Congo heamorrhagic fever, *Hyalomma marginatum*, Turkey

Symposium Wednesday, 11:00 128

Chikungunya, a threat for Europe? Opinion from an entomologist

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A major Chikungunya (CHIK) outbreak hit the islands of the Indian Ocean in 2005-2006. Surprisingly, the vector was *Aedes albopictus* instead of *Aedes aegypti*, the main vector. We orally infected *Ae. albopictus* collected in La Reunion Island in 2006 with two Chikungunya virus (CHIKV) which differ by a change from an Alanine (CHIKV E1-226V) to a Valine (CHIKV E1-226V) at the position 226 in the E1 glycoprotein. Disseminated infection rates assessed by immunofluorescent staining on head squashes of surviving females 14 days after infection, were at least two times higher with CHIKV E1-226V compared to CHIKV E1-226A. In addition, when analyzing the level of replication by quantitative RT-PCR, we showed that CHIKV E1-226V produced nearly 2 log more viral RNA than CHIKV E1-226A, the midgut playing a key role in limiting viral dissemination in the mosquito. Using a salivation technique to determine the amount of virus delivered by mosquitoes, we found that *Ae. albopictus* ensured a high replication of the virus which underwent an efficient dissemination as the virus became detectable in the salivary glands and the saliva at day 2 post-infection. Do the high levels of viral replication in *Ae. albopictus* lead to adverse effects impacting some mosquito life history traits? We found that CHIKV reduces sharply the survival of *Ae. albopictus* from La Reunion Island leading females to lay eggs earlier prior to death. Shortening the delay to laying could be a strategy for the host to compensate the negative effects of infection on female survival.

Keywords: *Aedes albopictus*, chikungunya, Indian Ocean, transmission

Symposium Wednesday, 11:30 129

Detection of Semliki Forest virus infection and induction of antiviral responses by the mosquito innate immune system

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Arboviruses are human and animal pathogens that are transmitted by arthropods such as mosquitoes. They replicate in both vertebrate and arthropod hosts, and thus need to employ strategies to deal with very different types of immunity. Mosquitoes are efficient at controlling arbovirus replication, and this is believed to be due to innate immune responses. Recent years have seen considerable progress in our understanding of arthropod innate immune responses to arbovirus infections, in particular the fields of RNA interference (RNAi) and antiviral signalling pathways. Activation of mosquito antiviral innate immunity requires recognition of molecular patterns, such as virus-induced dsRNA in the case of RNAi. I will discuss progress that has been made in understanding arbovirus detection, activation of antiviral mechanisms and consequences of infection for the mosquito cell in using the well-established Semliki Forest virus *(Togaviridae, Alphavirus*) model.

Keywords: Arbovirus-mosquito-innate immunity

Symposium Wednesday, 12:00 130

West Nile virus-host interactions: The poison is in the tail

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West Nile virus (WNV) is a highly pathogenic flavivirus (family *Flaviviridae*) that has claimed over a thousand lives in the USA alone since its unexpected emergence in New York in 1999. WNV circulates in a bird-mosquito cycle and is occasionally transmitted to humans by culicine mosquitoes. The 11 kb positive-stranded flavivirus RNA genome contains 5' and 3' untranslated regions (UTRs) and a single open reading frame encoding 10 viral proteins required for the viral life cycle. Flavivirus UTRs are highly structured RNAs involved in initiation of translation and viral RNA replication. In addition to full-length genomic RNA, a small (0.3-0.6 kb), subgenomic flavivirus RNA (sfRNA) derived from the 3' UTR is produced in abundant amounts in flavivirus-infected cells. Recently, sfRNA has been shown to be an important regulator of WNV pathogenicity, although its mode of

action remained unknown. Here, we show that conserved RNA structures with complex folding determine the production of sfRNA and that sfRNA has a novel activity in counteracting antiviral defense in cells of different origin.

Keywords: West Nile virus, mosquito, 3'UTR, RNA structure, pathogenicity

Symposium Diseases of Benefical Invertebrate
Wednesday 10:30-12:30
Nihat Turan 2

Pathogens of Pollinators: A Molecular Perspective

Organizer: Elke Genersch

Symposium Wednesday, 10:30 131

Transcriptome analysis of Honey Bee, Apis mellifera larvae infected with chalkbrood fungus

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We present here the experimental identification of honey bee genes that are differentially expressed in response to infection of honey bee larvae with the chalkbrood fungus, *Ascosphaera apis.* We used cDNA-AFLP Technology to profile transcripts in infected and uninfected bee larvae. From 64 primer combinations, over 7,400 transcriptionally-derived fragments were obtained. A total of 98 reproducible polymorphic cDNA-AFLP fragments were excised and sequenced, followed by quantitative real-time RT-PCR (qRT-PCR) analysis of these and additional samples. We have identified a number of differentially-regulated transcripts that are implicated in general mechanisms of stress adaptation, including energy metabolism and protein transport. Using a combination of cDNA-AFLP and qRT-PCR analyses, we were able to determine several key transcriptional events that constitute the overall effort in the honey bee larvae to fight natural fungal infection. Honey bee transcripts identified in this study are involved in critical functions related to transcriptional regulation, apoptotic degradation of ubiquitinated proteins, nutritional regulation, and RNA processing. We found that immune regulation of the anti-fungal responses in honey bee involves highly coordinated activation of both NF-κB signaling pathways, leading to production of anti-microbial peptides. Significantly, activation of immune responses in the infected bee larvae was associated with down-regulation of major storage proteins, leading to depletion of nutritional resources.

Keywords: Honeybee, fungal pathogen, *Ascosphaera apis*, transcriptional responses

Symposium Wednesday, 11:00 132

Nosema ceranae and *Nosema apis* – Comparative virulence

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Nosema apis and *Nosema ceranae* are intracellular microsporidian parasites infecting the midgut epithelial cells of adult honey bees (*Apis mellifera*). *N. ceranae* was considered to be restricted to the Asian honey bee, *Apis cerana*, but is nowadays a parasite found also in the European honey bee (*Apis mellifera*) across most of the world. Recent surveys and experimental work suggest that *N. ceranae* is a serious threat to the global beekeeping industry. It has been suggested that *N. ceranae* induces significantly higher mortality in honey bees than *N. apis*, but little is known about their comparative virulence. In this study, we used *in vivo* infection experiments to study the two parasites' different virulence (*i.e.* multiplication rate and infectivity). A qPCR was developed to elucidate within host competition between the two parasites using mixed infections. The outcome of the experiments indicates minor differences in infectious dose and multiplication rate between the two species. Moreover, the mortality caused by *N. ceranae* was not significantly higher than for *N. apis* and *N. ceranae* appeared to have no competitive advantage within host.

Keywords: *Nosema apis*, *Nosema ceranae*, virulence, honey bees

Symposium Wednesday, 11:30 133

European Foulbrood in the Molecular Era

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European foulbrood (EFB) is a severe bacterial brood disease caused by the Gram-positive bacterium *Melissocccus plutonius*. The disease is widely distributed worldwide and is an increasing problem in some areas. Although the causative agent of EFB was described for almost a century ago, many basic aspects of its pathogenesis are still unknown. Earlier studies have been hampered by insensitive and unspecific detection methods such as culture based techniques. Such methods are often unspecific and/or inadequate due to the secondary bacteria involved in disease development. Recent advances in molecular technology are making it increasingly easy to detect and characterize microbes, and nucleic acid detection technologies are quickly displacing the traditional phenotypic assays in microbiology. A number of protocols for qualitative and quantitative nucleic acid detection of *M. plutonius* have been published which enables studies of the occurrence and spread of the bacterium in honeybee colonies. Considering the increased incidence of EFB in some parts of the world, deeper knowledge of the epidemiology and virulence of the causative agent is of great importance for preventive actions to reduce or impede the spread of the disease.
This talk aim to present both historical data and new results obtained with modern molecular techniques and to synthesize present knowledge of this enigmatic honeybee disease. Future prospects and challenges will also be discussed.

Keywords: *Melissocccus plutonius*, European foulbrood (EFB)

Symposium Wednesday, 12:00 134

Molecular analysis of the honey bee pathogen *Paenibacillus larvae*

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Paenibacillus larvae is a rod-shaped and spore-forming Gram-positive bacterium causing American Foulbrood of honey bees. Recently, It was shown that the species *P. larvae* comprises different genotypes, which differ in virulence at the individual insect level and at colony level. *P. larvae* is able to infect honey bee larvae via the spores, but kills only the latter. The way of infection and killing is poorly understood. It has been shown that approximately 10 spores from virulent strains are sufficient to cause mortality. Genome sequencing of the *P. larvae* genotypes ERIC I and ERIC II was done by using 454-pyrosequencing. The obtained sequences were assembled and analyzed. The genome sizes were 3.8 Mb (ERIC I) and 4.3 Mb (ERIC II). The GC content of both genomes is approximately 45 %. Analysis of the genomes revealed a variety of potential genes, which might play an important role in niche adaption, infection and pathogenesis of *P. larvae*. These genes included gene clusters for synthesis polyketides, non- ribosomal peptide synthases, and toxins. In addition, approximately 80 genes encoding proteases and collagenases have been identified. We will present differences between the genotypes ERIC I and ERIC II and genome-derived models for infection and killing of honey bee larvae by *P. larvae*.

Keywords: Paenibaicllus larvae, honey bee, genome analysis

Contributed papers
Wednesday, 10:30-12:30
Fahri Kuran

FUNGI 3

Chairs: Drauzio Rangel and Ozlem Kalkar

Contributed Paper Wednesday, 10:30 135

Changing perspectives on the Entomophthorales: A new look at some of the oldest fungi

Richard A. Humber[1]; **Bo Huang[2]**; **Andrii J. Gryganskyi[3]**; **Rytas Vilgalys[3]**; **Kathie Hodge[4]**
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The recent phylogenetic realignment and reclassification of all fungi remains unfamiliar to many invertebrate pathologists but dramatically affected many fungal pathogens affecting insects. The order Entomophthorales is, arguably, the one group that remains the most enigmatic and least understood but is placed in the fungal family tree at the interface between flagellate and nonflagellate fungi. This comparatively small order occupied the most basal position among the nonflagellate fungi, immediately above the flagellate fungi. The phylum Zygomycota and class Zygomycetes are gone forever, and the subphylum Entomophthoromycotina still remains to be assigned to a new phylum. The correct placement of the genus *Basidiobolus* either with or apart from the Entomophthorales remains unsolved and perplexing and may depend ultimately on determining the correct phylogenetic linkages between the Neozygitaceae, Basidiobolaceae, and the remainder of the Entomophthorales. Even within the indisputable core of the order, the results of early phylogenetic studies have indicated that some major revisions of generic concepts will have to occur. *Conidiobolus*, as currently classified, is not monophyletic. There is support for some major divisions within the Entomophthoraceae, but perhaps not for all three subfamilies proposed by Keller and Petrini, and there may eventually be fewer zoophthoroid genera (with uninucleate, bitunicate conidia) than are accepted in the current morphologically based classification suggests. Unfortunately, the poorly known and rarely encountered genera of the families Completoriaceae and Meristacraceae remain completely unavailable for phylogenetic evaluation at this time.

Keywords: Phylogeny, revision, Ancylistaceae

Contributed Paper Wednesday, 10:45 136

Experiences in development of Beauveria bassinia for use in the IPM pest scarabs in Mexico

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Soil dwelling insects of the Family Scarabaeidae are pests of many crops in Mexico. The Laboratory of Pathology and Microbial Control of Insects (INIFAP, Uruapan Experimental Station, Michoacan) has focused on the use of entomopathogenic fungi for control of these pests and investigations have been carried out to find, evaluate and produce native strains of *Beauveria bassiana* and *Metarhizium anisopliae*. A bank of isolates from diverse regions of Michoacan has been formed and promising strains for control of pest *Phyllophaga* spp. identified. An IPM system using plant tolerance and application of entomopathogenic fungi has been developed for control of pest scarabs in maize. On the initiative of the local agricultural producers, with the support of the Federal and State Governments, a biofactory was designed and constructed in 2007-2008 for the production of entomopathogenic fungi in the municipality of Los Reyes. In 2009 local personnel were trained for mass production of fungi through solid substrate fermentation. Entomopathogenic fungi are now produced for application in programs of biological control promoted by the Rural Development Department, the State Committee of Plant Health and producers' organizations in cultures of blackberry, avocado, peach and maize. The biofactory is owned and administered by the organization "Productores

Agropecuarios por la Calidad" (PROCAL). INIFAP advises on the process of production, develops research and implements transfer of technology and scientific exchanges with national and international institutions.

Contributed Paper Wednesday, 11:00 137

The ATP-driven efflux pump is involved in *Isaria fumosorosea* resistance to carbendazim

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To explore possible mechanisms involved in the resistance of *Isaria fumosorosea* to carbendazim (methyl 2-benzimidazole carbamate; MBC), a highly MBC-sensitive wild-type strain (If116) were repeatedly mutated on the plates of MBC-inclusive medium. Linkage analysis of nine mutants showed that the α- and β-tubulin genes in the fungal genome were not related to the increased MBC resistance, which had no effect on heat sensitivity. The MBC-resistant mutants also displayed higher resistance to other antifungal drugs, such as tricyclazole, cymoxanil, thiophanate-methyl, azoxystrobin and iprodione, than the wild-type strain. MBC was found competing with the fluorescence stain RH123 in the If116 cell efflux. This indicates a possible involvement of target pleiotropic drug resistance (PDR) transporter in the fungal MBC resistance and was partially confirmed with less Rh123 accumulation in the cells of all the mutants with higher MBC resistance. To further elucidate the role of the PDR transporter in the fungal MBC resistance, a gene encoding an ATP-binding cassette transporter protein (IfTP1) was cloned from the cDNA library of the most MBC-resistant mutant (M1-47). Real-time PCR analysis showed that *IfTP1* expression in the wild-type strain was MBC-inductive, increasing with the drug concentration. Moreover, the gene was expressed in the mutants 2-10 fold more than in the wild-type strain. Conclusively, overexpression of the PDR transporter IfTP1 in cell membranes contributes largely to the increased MBC resistance in the mutants.

Keywords: *Isaria fumosorosea*, carbendazim, pleiotropic drug resistance

Contributed Paper Wednesday, 11:15 138

Characterization of a new mitochondrial Mn-SOD (BbSod3) from *Beauveria bassiana*

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Superoxide dismutase (SOD) may scavenge reactive oxygen species (ROS), which produces in eukaryotic cells under a stress and is harmful to biological *macromolecules*. A new mitochondrial Mn-SOD (BbSod3) was identified from *Beauveria bassiana*, an entomopathogenic fungus. The BbSod3-coding gene cloned from a *B. bassiana* strain was 879 bp in length, including two introns and three exons. The deduced protein consisted of 230 amino acid residues with the predicted molecular weight of 24.7 kDa and the isoelectronic point of 7.6. In online database search, the deduced BbSod3 showed 36-93% sequence identity to 75 Mn-SODs known from other fungi and was most close to the mitochondrial Mn-SOD of *Cordyceps militaris*. BbSod3 was found sharing all conserved amino acid residues with the Mn-SOD family, including the Parker-Blake signature, conserved pattern (DAWEHAYY) and four metal-binding residues. The first 35 amino acid residues of BbSod3 were predicted as a putative mitochondrium-targeting signal peptide with an export probability of 0.9987. This was further confirmed using transgenic strains expressing a fusion of the signal peptide and enhanced green fluorescent protein (eGFP). The eGFP was well expressed in intracellular reticular components, being in accordance with the red fluorescence of mitochondrial probe MitoTracker Red. A study is being undertaken to reveal the roles of this mitochondrial Mn-SOD and a cytosolic Mn-SOD (BbSod2) previously found in the same fungus.

Keywords: *Beauveria bassiana*, Mn SOD, Mitochondria

Contributed Paper Wednesday, 11:30 139

Analysis of a trehalose-6-phosphate synthase gene cloned from *Beauveria bassiana*

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Intracellular trehalose is likely involved in the thermotolerance of *Beauveria bassiana* because it may accumulate in the fungal entomopathogen under thermal stress. To explore possible mechanism involved in the fungal thermotolerance, we cloned from the fungal DNA a gene encoding trehalose-6-phosphate synthase (TPS) to convert uridine-5'-disphosphosglucose and glucose 6-phosphate to trehalose 6-phosphate, which can be dephosphorylated into trehalose by trehalose 6-phosphatase. The deduced 522-aa protein (58.3 kDa) from the cloned gene *BbTPS1* showed high sequence identity to other TPS from the fungi *Gibberella zeae* (92%), *Metarhizium anisopliae* (88%), *Neurospora crassa* (89%), *Magnaporthe grisea* (84%), and *Aspergillus fumigates* (75%). Three stress responsive elements (STREs) in a sequence pattern of AGGGG or CCCCT) were located in the 5'-untranslated region upstream of the initial codon ATG of open reading frame (ORF) of *BbTPS1*. Such STREs have proven to mediate transcriptional expression of the gene in response to different stresses and warrant more studies for their roles in the regulation of gene expression under multiple stresses.

Keywords: *Beauveria bassiana*, trehalose-6-phosphate synthase, thermotolerance

Contributed Paper Wednesday, 11:45 140-STU

The effect of the volume of medium on the growth and conidiation of *Pandora heteropterae* (Entomophthoraceae: Entomophthorales)

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Pandora heteropterae was grown on two different volumes of solid Sabouraud dextrose agar plus yeast (SDAY) in *vitro*. A number of different techiniques were used to measure the conidiation and mycelial growth over 31 days. The smaller volume yielded significantly more conidiation, while the larger volume had significantly more mycelial growth.

Keywords: Hemiptera, Lygus, sporulation

Wednesday, 10:30-12:30
VIEW POSTERS
Bacteria and COST 862
(Authors stand by posters when not in session)

12:30-14:00 **LUNCH at KTU SAHIL**

Senate Hall
13:00-14:00 **JIP Board Meeting**

Cross Divisional Symposium
Wednesday, 14:00-16:00
Hasan Turan
Viruses and Diseases of Beneficial Invertebrates
Viruses of Pollinators
Organizers: Nancy Ostiguy and Ivan Meeus

Symposium Wednesday, 14:00 141

Multiplex detection of viruses in bumblebees

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Bumblebees are commercially reared and transported worldwide mainly for pollination of greenhouse tomatoes. Indications of pathogen spillover from reared bumblebees to native bumblebees have been reported. The introduction of non-native pathogens is regarded as a threat for wild life biodiversity and could thus be contributing to current loss of bumblebees species worldwide. Cost-effective diagnostic techniques for detection of bumblebee pathogens is essential so that rearing facilities and governmental organizations can assure pathogen-free bumblebees by use of thorough sanitary controls. We will discuss: (i) the optimizing of a multiplex RT-PCR for the detection of the three viruses reported in bumblebees: *Acute bee paralysis virus* (ABPV), *Kashmir bee virus* (KBV) and *Deformed wing virus* (DWV). (ii) design of broad range primers in order to detect all viral variants. (iii) the controls needed according the International Organization for Standardization (ISO) in order RT-PCR technology can be used as a diagnostic technique. (iv) development and pitfalls of strand-specific RT-PCR based assays to detect replicative forms of bumblebee viruses.

Keywords: Acute bee paralysis virus, Kashmir bee virus, Deformed wing virus, Dicistroviridae, Iflaviridae, virus detection, multiplex RT-PCR, RNA-dependent RNA polymerase, bumblebees.

Symposium Wednesday, 14:30 142

Dicistroviruses in Honey Bees: An overview and future research direction

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The Dicistroviridae are a family of picorna-like viruses specific to insects. There are four dicistroviruses that naturally infect honey bees (*Apis mellifera*). One of these (*Black queen cell virus*) is one of the most prevalent viruses infecting honeybees. The virus persists through covert infection of adults and brood and clinical manifestations only affect developing queen pupae, leading to queen-cell abortion. The other three dicistroviruses (*Acute bee paralysis virus*, *Kashmir bee virus* and *Israeli acute paralysis virus*) are part of a species-complex with a widespread, but incidental prevalence in honey bee colonies and a predominantly sub-clinical etiology that contrasts sharply with the extremely virulent pathology encountered at elevated titres, either artificially induced or encountered naturally. These viruses are frequently implicated in honeybee colony losses, especially when the colonies are infested with the parasitic mite *Varroa destructor*. Here we review the historical and recent literature of these four viruses, covering history and origins; the geographic, host and tissue distribution; pathology and transmission; genetics and variation; diagnostics, and discuss these within the context of the molecular and biological similarities and differences between the viruses. We also briefly discuss three recent developments relating specifically to IAPV, concerning its association with Colony Collapse Disorder, treatment with siRNA and possible resistance.

Keywords: Dicistrovirus, honeybee, *Apis mellifera*, Colony Collapse, BQCV, ABPV, KBV, IAPV

Symposium Wednesday, 15:00 143

DWV – An interesting bee virus

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Reports in the recent past on a steady decline in honey bees in Europe, USA, and Japan caused great concern and initiated several studies to identify the underlying causes. From these studies it becomes more and more evident that the key players are pathogens and that among them it is mainly the ectoparasitic mite *Varroa destructor* (*V. destructor*) alone or in concert with certain viruses - which may also act independently - which are responsible for the observed colony collapses. One of the viruses heavily implicated in colony losses is *Deformed wing virus* (DWV). DWV can be detected in all live stages of the bee as well as in glandular secretions and faeces. DWV uses complex transmission routes within the bee population and between bees including transmission to and between different live stages. There is strong evidence that the outcome of an infection differs depending on the transmission route used. Therefore, we analyzed

the different transmission routes and the associated pathogenic effects to evaluate the impact of DWV for bees and colonies.

Keywords: Bee virus, DWV, pathogenic effects

Symposium Wednesday, 15:30 144

Recent advances in CBPV (Chronic Bee Paralysis Virus) study

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The Chronic bee paralysis virus (CBPV) is the aetiological agent of an infectious and contagious disease of adult honey bees known as chronic paralysis. Over the past few years, several outbreaks of trembling symptoms caused by CBPV have occurred in France. This led our laboratory to conduct studies in order to improve the knowledge on this agent and on the disease. Full-length nucleotide sequences for the two major RNAs of CBPV have been characterized, leading to the development of molecular diagnostic tools that can be used to detect and quantify genetically variable viral isolates. A two steps Real-Time PCR viral quantification technique allowed us to quantify the presence of the CBPV genome and its distribution, both within bees and within the hive. Significant high mortality rates were observed in France during the 2007, 2008 and 2009 beekeeping season. Bee samples from apiaries located in various parts of France were analyzed to evaluate the CBPV loads. Some surveyed apiaries presented high viral loads confirming the diagnosis of the chronic paralysis and highlighting the role of CBPV in bee mortalities. Moreover, detection of CBPV is reported for the first time in two species of ants (Camponotus vagus and Formica rufa). These results suggest that different modes of transmission of CBPV may occur and that different hosts may act as reservoir in the proximity of an apiary. The improved knowledge on the CBPV genome and variability, has allowed us to develop better tools to follow the disease and virus dissemination and ways of spread.

Keywords: Chronic bee paralysis virus (CBPV), Camponotus vagus, Formica rufa

Contributed papers
Wednesday, 14:00-16:00
Fahri Kuran

BACTERIA 3

Chairs: Christine Nielsen-Leroux and Ralf-Udo Ehlers

Contributed Paper Wednesday, 14:00
145-STU

***Bti* is not as safe! Tools for detecting *Bti* persistence and mosquito resistance in the field**

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Bti (*Bacillus thuringiensis* var. *israelensis*) is a bioinsecticide used worldwide against mosquito larvae. Thanks to no apparent persistence in the environment and a lack of evidence for resistance evolution in the field, this bioinsecticide is usually considered as efficient and safe. Nevertheless, decaying leaf litters showing high larval toxicity due to the persistence of *Bti* several months after a treatment was found in the French Rhône-Alpes region. Selecting an *Aedes aegypti* laboratory strain with this field-collected material for 20 generations led to a moderate increased resistance to *Bti* associated to a strong increased resistance to separated Cry toxins from *Bti*.
The persistence of *Bti* in the environment was investigated through the development of two new tools for monitoring *Bti* in field-collected samples: 1/ a specific quantitative PCR assay for quantifying *Bti* DNA in the environment, and 2/ an ELISA assay allowing to quantify each of the four main *Bti* toxins to evaluate the fate of each toxin. These tools were then used on field-collected samples and on samples from mesocosms artificially contaminated with *Bti* to investigate the factors influencing the persistence of *Bti* in the environment. To prospect for any cryptic development of *Bti* resistance due to its persistence in the French Rhône-Alpes region, mosquito larvae from various populations were sampled and bioassayed for resistance to commercial *Bti* mixture but also to separated Cry toxins (Cry4A, Cry4B, Cry11).
These results will be discussed in regard to *Bti* persistence in the environment and consequences on mosquito resistance and vector control strategies.

Keywords: *Bacillus thuringiensis* var. *israelensis*, qPCR, ELISA, Cry toxin, bioassay

Contributed Paper Wednesday, 14:15
146-STU

Bti proliferation in the environment: impact on the evolution of insecticide resistance

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Bacillus thurigiensis var israelensis (*Bti*) is a bacterio-insecticide widely used for the control of mosquito larvae in Europe. Until now, no stable *Bti* resistance has been detected in natural populations because the presence of 6 different toxins in *Bti* delays the emergence of resistance. Despite the supposed low persistence of *Bti* in the environment, we found highly toxic leaf litters containing large amounts of *Bti* spores in a mosquito breeding site long after *Bti* spraying, suggesting that *Bti* can proliferate in the environment, raising the concern of the evolution of resistance in treated populations. *Bti* produces crystal inclusions during sporulation that are mainly composed of four toxins that cause larval death. Until now, no stable *Bti* resistance has been detected in natural populations because the presence of 4 different toxins in *Bti* delays the emergence of resistance. A laboratory *Aedes aegypti* strain has been selected with toxic leaf litter,

and a resistance to toxic litter has appeared within a few generations. Moreover, the selected strain exhibit different levels of resistance against each *Bti* toxin tested separately, suggesting that different resistance mechanisms are at play. First, a comparative genome scan performed on the susceptible and resistant strains revealed several genomic regions putatively involved in *Bti* resistance. These regions were sequenced and located in the *Ae. aegypti* reference genome, allowing to identify candidate genes for *Bti* resistance. Then, transcriptomic analyses allowed the comparison of the transcription of more than 6000 genes between the susceptible and the resistant strain. The proliferation of *Bti* in mosquitoes breeding sites could influence the speed of development of *Bti* resistance in natural populations.

Keywords: *Bacillus thurigiensis* var *israelensis* (Bti), insecticide resistance, *Aedes aegypti*, genome scan, transcriptomic

Contributed Paper Wednesday, 14:30 147

Increase in midgut microbiota load increases tolerance to *Bacillus thuringiensis*

Patricia Hernández-Martínez[1]; **Bahram Naseri**[1]; **Gloria Navarro-Cerrillo**[1]; **Baltasar Escriche**[1]; **Juan Ferre**[1]; **Salvador Herrero**[1]
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The insect immune system is comprised of both humoral and cellular components that are mobilized in response to parasitic or pathogenic infections. Recent evidence suggests that although inducible, the insects can prolong the activation of the immune response and even transmit the immune status trans-generationally. This phenomenon, also known as immune priming, has a positive impact on the primed insects that become more tolerant to the pathogen than the non-primed insects. Using the lytic zone assay as a measure of the immune status of the individual larvae, we have selected for a colony of the lepidopteran *Spodoptera exigua* with high levels of immune activity in the absence of external challenging with bacteria. Immune activated insect showed characteristics that are typical reported for immune primed insects, such as increased tolerance to a pathogen such as *Bacillus thuringiensis*, fitness-cost associated to the immune status, and maternal transmission of the immune status. Additional analysis revealed that the selection for the immune activated insects was based on the selection of insects carrying a higher bacterial load in the midgut suggesting that activation of the immune system in *S. exigua* may not only occur as consequence of the immune priming but also from an increase in midgut microbiota load.

Keywords: Immune priming, microbiota, *B. thuringiensis*, *Spodoptera exigua*

Contributed Paper Wednesday, 14:45 148

Influence of population density of *Xenorhabdus bovienii* and *X. nematophila* on the development of their symbiotic nematodes *Steinernema feltiae* and *S. carpocapsae* in monoxenic liquid cultures

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Xenorhabdus spp. are the major food source for EPN of the genus Steinernema. Commercial production is done in monoxenic liquid cultures inoculated with the symbiotic bacteria and a day later with nematode third stage dauer juveniles (DJs). Results on the nematode population dynamics indicated that bacterial cells at a higher density induce not only a higher DJ recovery, but also accelerate the development of recovered juveniles to adults. Alternative nematode developmental pathways result in either DJs or an additional adult generation. The development to adults is a response to higher bacterial cell density, to DJs by depleting food resources. The development is thus driven by the density of the symbiotic bacteria culture.

Keywords: Symbiosis

Contributed Paper Wednesday, 15:00 149

Ultrastructural changes in the gut of adult flies after *Brevibacillus laterosporus* ingestion

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We studied the anatomo-pathological effects in the gut of adult flies after ingestion of *Brevibacillus laterosporus* spores. After dissection and fixation, gut tissues were analysed under both scanning electron microscopy (S.E.M.) and transmission electron microscopy (T.E.M.). Comparisons between *in vivo*-treated and non-treated (control) fly specimens were conducted over-time in order to highlight the increasingly extensive pathological alterations.
Midgut epithelial cells of treated flies display general disruption of the microvillar structure, mitochondria alterations, increased population of lysosome-like structures, ribosomes strongly reduced in numbers and rough endoplasmic reticulum rather upset, apical cytoplasm vacuolization which eventually leads to the cell rupture, basal labyrinth completely disorganized. Disruption was associated also with midgut muscular sheath and connective tissue.
These observations suggest that the pathogenic activity of this *B. laterosporus* strain for *M. domestica* is a toxin-mediated process reminiscent of the mechanism of action of *B. thuringiensis* δ-endotoxins.

Keywords: Electron microscopy, gut alterations, *Brevibacillus laterosporus*, house fly

Contributed Paper Wednesday, 15:15 150

Oral insecticidal activity in biocontrol pseudomonads

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FitD from *Pseudomonas fluorescens* CHA0 is a novel insect toxin in root-colonizing pseudomonads. Previous sequence analyses suggested a horizontal gene transfer from the entomopathogenic bacterium *Photorhabdus luminescens* with subsequent rearrangements of the cluster harbouring the *fitD* toxin gene. An extensive screening, followed by phylogenetic analyses shows that the occurrence of *fitD* is restricted to a small subgroup of pseudomonads producing the antifungal compound 2,4-diacetylphloroglucinol and exhibiting biocontrol properties against fungal root diseases. A series of feeding and contact assays demonstrated that these bacteria exhibit strong oral activity against larvae of the lepidoptera *Spodoptera littoralis*, *Heliothis virescens* and *Plutella xylostella*, all important insect pests of agricultural crops. Derivatives of *P. fluorescens* CHA0 with various defects in Fit locus expression exhibited altered toxicity towards the tested larvae. Our experiments show for the first time the lethal oral activity of fluorescent pseudomonads when ingested at a sufficient cell density. These finding add value to the biocontrol potential of plant-beneficial pseudomonads, specifically towards their potential use in insect pest management.

Keywords: Insecticidal toxin, oral activity, biocontrol pseudomonads

Contributed Paper Wednesday, 15:30 151

Insecticidal effect of *Bacillus thuringiensis* Berliner ssp. *tenebrionis* (Bacteria: Bacillaceae), on partially treated wheat and maize surfaces, against larvae of *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae), *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) and *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae)

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A formulation of *Bacillus thuringiensis* Berliner ssp. *tenebrionis* (Bacteria: Bacillaceae) was evaluated in laboratory bioassays as a surface treatment in wheat or maize columns to control larvae of *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae), *Oryzaephilus surimamensis* (L.) (Coleoptera: Silvanidae) and *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Cucujidae). The insecticide was applied at 35 g of wheat or maize placed in a vial or to the upper one half, one fourth, or one eighth layer of the grain, and insects were either added to the vials before or after the grain placement. *T. confusum* larvae were the most susceptible, followed by *C. ferrugineus* and *O. surinamensis.* However, mortality varied according to the type of insect placement. Hence, insects that were present in the grain column under the treated surface (in the untreated part of the grain) had lower mortality than insects that were present in the top of the grain column. Mortality was increased with the increase of the treated layer. Moreover, efficacy was reduced on maize in comparison with wheat, for all species tested. Our study demonstrate the potential of using *B. thuringiensis* as a "top dressing" approach on grain bulks, but its efficacy is notably affected by the commodity, the size of the treated layer and the target species.

Keywords: *Bacillus thuringiensis* ssp. *tenebrionis*, *Tribolium confusum*, *Oryzaephilus surinamensis*, *Cryptolestes ferrugineus*

Contributed Paper Wednesday, 15:45 152

Human exposure to airborne *Bacillus thuringiensis kurstaki* HD1 and other bacteria in greenhouses and vegetable fields

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Knowledge of the natural occurring bioaerosols in horticulture environments can aid in the risk assessment of introducing new organisms into the working environment through biocontrol agents.
Few studies have quantified vegetable growers' exposure to *Bacillus thuringiensis* (*Bt*) from biocontrol products during working hours. In this study we have collected air samples from greenhouses and open fields obtained from workers breathing zone and quantified the presence of *Bt* and other mesophilic bacteria. Air samples were analysed by plate counts, and total counts with a microscope. Isolates resembling HD1 were identified by PCR analysis. We found that greenhouse workers, especially in cucumber production (with no use of *Bt*), were exposed to high concentrations of naturally occurring bacteria (reaching 1,100,000 cfu/m^3). *Bacillus cereus*-like bacteria were seldom detected in this system and only in concentrations close to the detection level (80 cfu/m^3). Also in open fields the exposure to natural occurring bacteria was higher than the exposure to *Bacillus cereus*-like bacteria. HD1-like bacteria were only detected in environments where Dipel® was used. In a greenhouse with Dipel® treated tomato plants the growers' exposure to airborne HD1-like bacteria reached 5,300 cfu/m^3 and 1,400 cfu/m^3 during harvest and clearing of old plants, respectively. Thus, the presence of airborne bacteria in vegetable production might have a greater influence on growers' health, due to the high concentrations found, than applied biocontrol strains.

Keywords: Human exposure, *Bacillus thuringiensis*, vegetable production, bacteria

Contributed papers

Wednesday, 14:00-16:00
Nihat Turan 2

NEMATODES 2

Chairs: Jeanne de Waal and I. Alper Susurluk

Contributed Paper Wednesday, 14:00 153

Analysis of community composition, diversity and function of nematodes in the rhizosphere soil of replanted and non-replanted peach orchards

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The study related on the analysis of community composition, diversity and function of nematodes in the rhizosphere soil of replanted peach orchards distributing in 3 different regions of Beijing, China based on the survey data from April 2006 to December 2009. The result showed that the bacterivores and herbivores were the main trophic groups in all soil samples. The nematodes in the genera of *Acrobeloides*, *Rhabditiss*, *Eucephalobus*, *Aphelenchus* and *Tylenchus* were dominant ones in both replanted and non-replanted peach orchards. The nematodes in the genus of *Diploscapter*, *Plectus*, *Monhystera*, *Wilsonema*, *Rotylenchus*, *Helicotylenchus* and *Tylenchorhynchus* could be found only in the soil of replanted peach orchards. There were no typical nematodes could be found only in the non-replanted peach orchards. The indexes of Shannon-Wiener diversity index (*H'*), Species Richness (*SR*) and Simpson Evenness (*E*) were no significant difference in both kinds of orchards. The same situation happened with the Structure index and Enrichment index. However, in the replanted orchards, the density of plant parasitic nematodes (PPNs) in general was 2-5 folds of that in the non-replanted orchards. The density of PPNs could be reduced 50% down by the nematodes of *Rhabditis hailarensis*, isolated by the authors.

* The study was supported by the Ministry of Science and Technology of China 863 Project: "Soil Technique of Nematode Repair Biological Pollution" (2006AA06Z354) and National Science and Technology Support Project: "Production Technology of High Efficiency Standard on Main Fruit Trees" (2008BAD92B08).

Keywords: Nematode community composition, diversity, abundance, function, replanted peach orchard

Contributed Paper Wednesday, 14:15 154

Scavenging extends the host range of entomopathogenic nematodes (Nematoda: Steinernematidae)

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Entomopathogenic nematodes have been considered to be obligate parasites or pathogens of insects. Recently, several studies have shown EPNs being able to colonise and multiply in freeze-killed Galleria mellonella suggesting that they can use scavenging as an alternative life strategy. In present study, living and freeze-killed natural and laboratory hosts, with different susceptibility to entomopathogenic nematodes, were exposed to the larvae of Steinernema affine and S. kraussei in two different experimental arenas (Eppendorf tubes, Petri dishes), and the success of the colonisation and eventual progeny production were observed. Both nematodes were able to colonise both living and dead larvae of Galleria mellonella (Lepidoptera) and adult Blatella germanica (Blattodea) even though the progeny production in dead hosts was lower on average. Living carabid beetles, Poecilus cupreus, and elaterid larvae (Coleoptera) were resistant to the infection, however, both nematodes were able to colonise and multiply in several dead P. cupreus and in a majority of dead elaterid larvae.

In another set of experiments, three types of an experimental host G. mellonella (living, freeze killed and killed by S. kraussei) were exposed to S. affine and its invasion rate was observed. Highest invasion rate was recorded in nematode killed hosts, followed by freeze killed and living ones and in all hosts, S. affine successfully reproduced.

Our results suggest that by scavenging, EPNs can utilise cadavers of insects that are naturally resistant to EPN infection, and so broaden their host range. I also appears that hosts or cadavers already infected by another suitable EPN can be another good alternative to standard infection. The advantage of invading such hosts may be no immune response and already developed population of bacterial symbiont.

Keywords: Entomopathogenic nematodes, Steinernema, scavenging

Contributed Paper Wednesday, 14:30 155-STU

Potential of entomopathogenic nematodes for the control of *Phlyctinus callosus* (Schönherr) (Coleoptera: Curculionidae)

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Several endemic entomopathogenic nematode isolates were evaluated for their potential use as biological control agents for *Phlyctinus callosus*, which is a pest insect on deciduous fruit. The susceptibility of *P. callosus* larvae and adults to entomopathogenic nematodes was screened in the laboratory at a concentration of 400 infective juveniles (IJ) per insect after four days. The SF41 isolate of *H. zealandica* was selected as the most promising isolate against the larvae and *H. bacteriophora* (SF134) against adults. The effect of concentration, temperature, vertical movement in river sand and sandy loam soil, and the biology of *H. zealandica* in *P. callosus* larvae were also investigated in the laboratory. The LD50 and LD90 values after a four day incubation period were 96 and 278 IJ/50 µl, respectively. A higher (95.2%) percentage mortality rate was obtained with the sandy loam soil, than with the use of river sand (77.5%). *Heterorhabditis zealandica* could successfully complete its life cycle in 6th instar P. callosus larvae. The current study showed that *P. callosus* larvae are

suitable hosts for *H. zealandica*. The selected isolate might be used to control *P. callosus* larvae in an integrated pest management programme.

Keywords: *Phlyctinus callosus*, Entomopathogenic nematode, *Heterorhabditis zealandica*

Contributed Paper Wednesday, 14:45
156-STU

European Earwig (*Forficula auricularia*) as a novel host for the entomopathogenic nematode *Steinernema carposcapsae*

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The natural history of many entomopathogenic nematodes remains mysterious, despite their wide commercial availability as biological control agents. In close ecological proximity to the ambushing entomopathogenic nematode, *Steinernema carpocapsae*, the introduced European earwig (*Forficula auricularia*) forages and burrows on the soil surface. Few studies have examined earwigs' susceptibility to entomopathogenic nematodes, however. In the laboratory, *S. carpocapsae* killed *F. auricularia* at a dose of 226 nematodes/host and new infective juveniles emerged approximately 10 days later. After exposure to *F. auricularia* cuticle, *S. carpocapsae* were attracted to CO_2 at rates comparable to *Galleria mellonella*, indicating good host quality. If earwigs naturally encounter entomopathogenic nematodes then we would expect them to have evolved anti-parasite defenses. Indeed, we found that earwigs exposed to *S. carpocapsae* cleaned and scratched their front, middle and back legs significantly more than controls ($P<0.001$). Earwig susceptibility also depended on body size with significantly higher mortality rates seen in larger earwigs ($P = 0.028$). We also report on the reproductive potential of earwigs as hosts as well as the LC_{50} for both large and small earwigs. Coupled with previous field data, these findings lead us to suggest that *F. auricularia* may be a natural host for *S. carpocapsae*.

Keywords: biological control, entomopathogenic nematode

Contributed Paper Wednesday, 15:00 157

Modifying citrus planting sites promotes conservation biological control of the root weevil *Diaprepes abbreviatus* by entomopathogenic nematodes

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Diaprepes abbreviatus is a major root pest of citrus on the shallow, fine sand soils of eastern Florida but less so in orchards on the deep, coarse sand of central Florida. The diversity of entomopathogenic nematodes in east coast orchards is usually restricted to a single species (*Heterorhabditis indica*), whereas 3-5 species typically occur in central Florida and cause mortality of buried sentinel weevil larvae as high as 80% week^{-1}. Some growers on the east coast plant trees in oversized planting holes filled with coarse sand to improve drainage. Four EPN species from central Florida were introduced into these coarse sand 'mesocosms' in April 2006 to assess persistence and biological control potential. Sentinel weevil mortality in plots augmented with *Steinernema diaprepesi* was >80% week^{-1} four and six months after augmentation, twice that in untreated plots. Augmentation with *S. riobrave* and *H. zealandica* did not increase weevil mortality although both were detected for up to 12 months. During 48 months, mortality of sentinel weevils averaged 73% in *S. diaprepesi* plots compared to 33-51% in control or other augmented plots. *S. diaprepesi* persisted in 40% of augmented plots 4 years after augmentation but was never detected in adjacent plots. In a second experiment, trees in native sandy loam soil were half the size of adjacent trees in coarse sand mesocosms one year after planting. Increased canopy density of trees in coarse sand compared to sandy loam was greater in plots augmented with *S. diaprepesi* (but not *S. riobrave*, *H. indica*, or *H. zealandica*), than in unaugmented plots.

Keywords: Entomopathgenic nematodes, conservation biological control, citrus, soil type

Contributed Paper Wednesday, 15:15
158-STU

The Response of *Phasmarhabditis hermaphrodita* (Nematoda: Rhabditidae) and *Steinernema feltiae* (Nematoda: Steinernematidae) to volatile and water soluble cues

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The slug parasitic nematode, *Phasmarhabditis hermaphrodita*, is capable of kiling many slug and snail species, such as *Deroceras reticulatum*, which is the most widespread slug pest in the world. Its life cycle is very similar to the genera *Steinernema* or *Heterorhabditis*. It is known that entomopathogenic nematodes use various cues, for example faeces, carbon dioxide or other contact and volatile cues. In this work I tested the response of *P. hermaphrodita* and *S. feltiae* to different slug and insect cues (*D. reticulatum* and *Galleria mellonella*). Tests were performed on agar plates and in olfactometers. It was found that both nematodes react actively to relevant cues on agar plates but they response very weakly or even not at all to the same cues in olfactometers. These results corespond with the statement that nematodes react badly to volatile cues. However, there are some exceptions. e.g. *P. hermaphrodita*, under the test conditions, tends to avoid space with the occurrence of volatile cues from dead *D. reticulatum*. Similar, but much weaker reaction was observed in the experiments with *S. feltiae*.

Keywords: *Derocera reticulatum*, *Galleria mellonella*, olfactometer, chemoattraction, entomopathogenic nematode

Contributed Paper **Wednesday, 15:30**
159-STU

Development time and survivorship of *Deladenus siricidicola* (Tylenchida: Neotylenchidae) on different strains of *Amylostereum areolatum* (Russulales: Stereaceae)

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The invasive pine woodwasp *Sirex noctilio* (Hymenoptera: Siricidae) can infest and kill healthy pine (Pinus spp.) trees. It owes this success to a symbiosis with a tree pathogenic fungus, *Amylostereum areolatum*, which the woodwasp injects into pine trees during oviposition. The parasitic nematode *Deladenus siricidicola* has been used successfully for biological control of *S. noctilio* in the Southern Hemisphere. *D. siricidicola* has a parasitic form and a fungal-feeding form, the latter of which is used for mass production. Because a different strain of *A. areolatum* is used by native woodwasps in North America, *D. siricidicola* used for control of *S.* noctilio in the United States might grow on it at a different rate, affecting control. This study focused on the hatching rate of eggs of *D. siricidicola*. Eggs were then inoculated onto plates of several strains of *A. areolatum*, which grow at different rates, to test the nematode's ability to grow on the strains. It was found that too much fungal growth results in nematodes being overgrown by fungus, and too little fungal growth results in nematodes running out of food. Thus, presence of other strains of *A. areolatum* could affect the effectiveness of *D. siricidicola* in the United States.

Keywords: *Sirex noctilio*, *Deladenus siricidicola*, *Amylostereum areolatum*

Wednesday, 14:00-16:00
VIEW POSTERS
Fungi (Authors stand by posters)

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

Contributed papers
Wednesday, 16:30-18:00
Nihat Turan 1

DISEASES OF BENEFICIAL INVERTEBRATES 1

Chairs: Grant Stentiford and Ibrahim Cakmak

Contributed Paper **Wednesday, 16:30 160**

Specialized parasite (*Varroa destructor*) and hygienic behavior of honey bees (*Apis mellifera*)

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Varroa destructor has been the main factor for *Apis mellifera* colony losses despite three decades of intensive research aimed at controlling this pest. In addition to the effect of the mite itself on honey bees, *V. destructor* is a vector for a number of viruses that also significantly affect honeybee population survival (Sumpter and Martin 2004, Kevan et al. 2006).Thus, this specialized parasite of honey bees, and when infesting *Apis mellifera* kills its host colony in just one to two years in Northwest of Turkey. However, on its original host, the Eastern honey bee (*Apis cerana* F.), infestation with *V. destructor* has minimal effect, which shows that a more balanced parasite-host relationship is possible (Rath 1999). Furthermore, *V. destructor* is not a major problem in Africanized bees (*A.m. scutellata*) due to worker behaviors. Thus, the hygienic-grooming model for innate resistant to *Varroa* gains additional credence as a potential solution to *Varroa* parasitism (Guzman-Novoa et al.1999, Mondragon et al. 2005). The possible mechanisms are Varroa Sensitive Hygiene, grooming behaviour, biting, damaging mites, excluding infested worker bees from the colony, or repellent odour of brood and or young workers. The honey bee as a social insect may have a combination of collective behavioural, cellular, and humoral defence mechanisms for parasites (Wilson-Rich *et al.*, 2008). One or possibly combination of these mechanisms might play a role in resistance to *V. destructor*, and all of these possibilities need to be tested.

Keywords: *Varroa destructor*, *Apis mellifera*, Honey bee, Hygienic behaviour, Resistance

Contributed Paper **Wednesday, 16:45 161**

Assessment of the environmental impact of a "stacked" Bt-maize line with multiple resistances on non-target arthropods

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The introduction of the Western Corn Rootworm (*Diabrotica virgifera virgifera*) to Europe and its ongoing spread establishes this chrysomelid as a potential future problem for maize cultivation. Together with the European corn borer (*Ostrinia nubilalis*) there will now be two serious pests in most European maize growing regions. This threat can be answered by the deployment of "stacked" maize lines combining multiple *Bacillus thuringiensis* genes conferring pest resistance like MON89034 x MON88017, with the three *Bt* proteins Cry3Bb1 (against *Diabrotica*), Cry2Ab2 and the synthetic Cry1A.105 (against *Ostrinia*). To assess the non-target impact of this stack, we perform a field release experiment over three seasons. To assess possible *Bt* effects and varietal differences, we compare the genetically modified line, its near isogenic line (with and without a soil insecticide treatment for *Diabrotica* control) and two conventional maize cultivars
The choice of non-target organisms (NTO) is based on experiences gained in previous field release experiments, the exposure of the NTOs and their representativeness for the biocoenosis. Herbivorous arthropods of the herb layer (e.g. plant bugs, plant- and leafhoppers, leaf-beetles), flower-visiting arthropods (e.g. thrips), and their predators (e.g.

predatory bugs and lady-beetles) were chosen and are sampled using different methods. Selected organisms are assessed for their internal content of the different *Bt* proteins with ELISA. Studies of the fate of the *Bt* proteins in the plant bug *Trigonotylus caelestialium* as a model, will add to the impact assessment. Data of the first two years of this project will be presented.

Keywords: Non-target organisms, Bt maize, environmental risk assessment, *Trigonotylus caelestialium*

Contributed Paper Wednesday, 17:00
162-STU

Potential effects of the *Diabrotica* virgifera *vir.* specific Cry3Bb1 on the ground beetle *Poecilus cupreus* (L.) evaluated in a full life cycle test

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The cultivation of genetically modified maize varieties expressing *Bt* proteins may pose a risk for beneficial non-target organisms. The stepwise approach of an environmental risk assessment (ERA) starts with laboratory bioassays looking for potential effects of the expressed *Bt* proteins. For this purpose, the establishment of standardized test procedures with the capability of reproducible results is needed.
Ground beetles (Coleoptera: Carabidae) are common epigeal predators in the maize field biocoenosis, representing an important agent of integrated pest management, and are therefore in the focus of research. The breeding of the carabid *Poecilus cupreus* (L.) is well described because this species is used in the environmental risk assessment of pesticides (Heimbach et al. 1995).
The test procedure used in pesticide evaluation was adapted for the testing of the *Bt* proteins. The newly developed test design and results of a bioassay with 300 carabid individuals fed with the coleopteran specific Cry3Bb1 are presented.

Keywords: Carabidae, ground beetle, cry3Bb1, bioassay

Contributed Paper Wednesday, 17:15 163

RNAi at work: Targeting invertebrate pests and demonstrating effective RNAi protection from pathogenic diseases in HoneyBees

Gal Yarden[1]; **Eitan Glick**[1]; **Ilan Sela**[2]; **Eyal Maori**[2]; **Wayne Hunter**[3]; **Jay Evans**[3]; **Nitzan Paldi**[1]; **Eyal Ben**[4]
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RNA interference (RNAi) applications for invertebrates present two types of large scale opportunities; targeted pest control and beneficial organisms' health. RNAi-based solutions are environmentally friendly and target specific.
Beeologics established an affordable large scale production method for production of double stranded RNA (dsRNA), the initiator molecule in RNAi reactions. Remebee™ is the company's leading dsRNA product, which is homologous to honeybee viral sequence. The exogenously supplied Remebee mimics the natural dsRNA intermediate involved in viral replication within the honeybee cells. In large scale field trials, the gene silencing mechanism induced by Remebee that was fed to the bees was shown to be highly effective in preventing honeybee mortality from the Israel Acute Paralysis Virus (IAPV).
The Colony Collapse Disorder (CCD) phenomenon is still not fully understood or agreed upon; however, there is a strong consensus some specific pathogens and pests are major contributing factors to colony losses. Viruses in general and IAPV in particular, microsporedia such as the Nosema Ceranae and the Varroa mite are considered the top three contributors to the phenomena. Beeologics has developed a generic technology platform which is utilized to introduce a full RNAi product-line targeting all viruses, Nosema and Varroa. Moreover, future products for other agriculture and veterinary applications are currently being set-up.
Any invertebrate organism whose genome has been fully or even partially sequenced may be amenable to RNAi application opportunities. Basic elements of the new products design including: mechanism of action, large-scale production, regulation and ongoing development projects will be discussed.

Keywords: RNAi, honeybee, virus, nosema, varroa, targeted pest control

Contributed Paper Wednesday, 17:30 164

Distribution and variation of *Nosema bombi* in North American bumble bees

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Several bumble bee species in North America appear to have undergone rapid declines in abundance. *Bombus franklini* is potentially extinct; *B. occidentalis* and *B. affinis* currently are experiencing extensive range reductions; and a shift in the range of *B. pensylvanicus* indicates a similar trend. In an unprecedented survey, we collected and evaluated pathogen load in 9,903 specimens of 36 *Bombus* species in 38 states, but focused on *Nosema bombi infections in* two putatively declining species, *B. occidentalis* and *B. pensylvanicus*, and four stable species. *Nosema bombi* was recovered from 2.9 % of all collected specimens. It was present in bumble bee populations in 25 of the surveyed states, predominantly in the declining *B. occidentalis* (37 %) and *B. pensylvanicus* (15.2 %). *N. bombi* infections occurred in these two hosts in more than 40 % of surveyed sites, significantly more than for other hosts. Recovered *N. bombi* isolates were genetically identical to European strains. The only variation in the pathogen among host species was found in the ITS region of the rRNA gene, indicating the presence of multiple alleles. These findings support the hypothesis that *N. bombi* is Holarctic in distribution, but susceptibility to the pathogen varies among *Bombus* species.

Keywords: Bombus, decline, distribution, *Nosema bombi*

Contributed Paper Wednesday, 17:45 165

Involvement of Deformed Wing Virus (DWV) and Varroa Destructor Virus in the deformed wing syndrome of the honey bee

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Recently we performed a country- wide screen for viruses affecting honey bees in Israeli apiaries that revealed a high incidence of the Deformed Wing Virus (DWV) and the *Varroa Destructor* Virus (VaDV). In order to understand better the relationship between the presence of the above viruses and the deformed wing pathology, we performed qualitative and quantitative analysis of DWV and VaDV replication in individual bees from apiaries with overt infections, utilizing RT-PCR and quantitative Real-Time PCR. Also, we followed up the presence of the above viruses by immunoblot analysis of the viral capsids in the infected bees. Our data indicate that both viruses may be able to induce the deformed wing pathology depending upon their ability to replicate efficiently in the bee host.

Keywords: DWV, VaDV, Honey bee

Contributed papers
Wednesday, 16:30-18:00
Nihat Turan 2

NEMATODES 3

Chairs: S. Patricia Stock and Ramazan Canhilal

Contributed Paper Wednesday, 16:30 166

Influence of humidity, water application volume and a formulation on the control potential of the entomopathogenic nematode *Steinernema feltiae* on overwintering larvae of the codling moth *Cydia pomonella*

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Codling moth (*Cydia pomonella* L.) is a serious pest of pome fruit. Diapausing cocooned larvae overwinter in cryptic habitats in the soil around or the bark of infested trees. The entomopathogenic nematode *Steinernema feltiae* (Rhabditida: Steinernematidae) is used to control diapausing codling moth larvae. In summary, following recommendations can be drawn from the results: 1. Application should be against cocooned larvae, because they are more susceptible. 2. Relative humidity should at least be at 80% during application and few hours after application. 3. The lower the relative humidity, the high should be the application volume of water. 4. The surfactant-polymer-formulation should be used, particularly when suboptimal environmental conditions cannot be expected.

Keywords: Codling moth, *Steinernema feltiae*

Contributed Paper Wednesday, 16:45 167

Virulence of South Carolinian Heterorhabditid Isolates to *Spodoptera exigua* (Lepidoptera: Noctuidae)

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The beet armyworm, *Spodoptera exigua* (Hubner) (Lepidoptera: Noctuidae) is an important polyphagus pest of cultivated crop primarily in the tropical and subtropical regions. We aimed to compare the virulence of nine heterorhabditid isolates from South Carolina to the beet armyworm with two known heterorhabditid nematodes, *Heterorhabditis bacteriophora* Poinar HP88 and Hb strains under laboratory conditions. Nine nematode isolates studied were *H. bacteriophora* Poinar CFG, CFM, MF, SMP, PD and WPS strains, *H. zealandica* Wouts CHR and EDS strains, and *H. megidis* Poinar, Jackson and Klein LEX strain. The Petri plate bioassay procedure was used to evaluate the susceptibility of the beet armyworm larvae to heterorhabditids at concentrations of 10, 25, 50, and 100 infective juveniles (IJs) per larva. Mortality was recorded daily for four days and the bioassay was repeated two times. The mortality induced by nematodes increased, typically with increasing numbers of nematodes per larva and at the final count, it was 53.57-100, 72.02-100, 79.76-100, and 92.8-100% for the concentrations of 10, 25, 50, and 100 IJs, respectively. Overall, the nematodes that produced the highest mortality were *H. bacteriophora* HP88 and WPS strains, *H. zealandica* EDS and CHR strains, and *H. megidis* LEX strain. *Heterorhabditis megidis* LEX strain differed than others by having 100% mortality in all of concentrations. Our results suggest that *H. bacteriophora* WPS strain, *H. zealandica* EDS and CHR strains, and *H. megidis* LEX strain should be studied further as potential biocontrol agents of the beet armyworm and other similar lepidopters.

Keywords: Heterorhabditis, *Spodoptera exigua*, biological control

Contributed Paper Wednesday, 17:00 168

Field trials with entomopathogenic nematodes for the control of false codling moth (*Thaumatotibia leucotreta*)

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False codling moth, *Thaumatotibia leucotreta*, is the most important pest of citrus in South Africa. It is an endemic species and restricted to countries south of the Sahara. Currently, none of the control measures used against false codling moth are directed at the soil stage, which lasts up to 20 days and longer depending on temperature. The final instar false codling moth larvae burrow a few millimetres into the soil to spin a cocoon. These subterranean stages of the life cycle –

prepupae, pupae and emerging moths – offer a window of opportunity for applying entomopathogenic nematodes. Nematodes as a control measure against false codling moth have not previously been investigated. They can fill an important control niche in spring, early summer and autumn. They can even be applied after harvest, when traditionally no control measures are used in citrus orchards. Field trials were conducted in a high density planted Satsuma mandarin orchard, with a sandy loam soil at mean ambient temperatures of between 17-25ºC. Nematodes (*Heterorhabditis zealandica*, *H. bacteriophora* and *Steinernema khoisanae*) were applied with hand held sprayers at different concentrations. Final instar larvae were contained in orchard soil in 40 mesh cages, buried next to each treatment tree. After two to six days the cages were retrieved and larvae and pupae washed from the cages and infection determined. Control of up to 80% was obtained by using only 10 IJ/cm^2 of *H. zealandica*. Thirty five days after application, 70% control was still achieved.

Keywords: *Thaumatotibia leucotreta*, codling moth, field trials, entomopathogenic nematodes

Contributed Paper **Wednesday, 17:15**
169-STU

Mulching Madness- Evaluating the use of mulches in conjunction with entomopathogenic nematodes for the microbial control of codling moth

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Mulching is becoming increasingly popular worldwide, due to advantages associated with this practice such as increasing overall biodiversity in agroecosystem environments. The use of mulches in conjunction with natural enemies as a pest management strategy has only recently been exploited, such as applying entomopathogenic nematodes together with a mulch (which in turn protects nematodes from environmental extremes) to control codling moth, *Cydia pomonella* (L.) (known to diapause in mulches beneath trees). A study was undertaken to investigate the use of *Heterorhabditis zealandica* in conjunction with different mulch types (pine chips, wheat straw, pine wood shavings, blackwood and apple wood chips) in laboratory and field experiments. Aspects investigated included: incubation time, reproductive rate of nematodes inside larvae at low temperatures, nematode mobility within mulches, environmental conditions and residual activity of nematodes in mulches post-treatment. Results obtained illustrated the baseline requirements fundamental to successful applications, such as using mulch which is practical (straw and apple chips), whilst ensuring optimum humidity for at least three days, moderate temperatures and no direct sunlight exposure to ensure the survival and subsequent activity of nematodes. Accordingly, these results can assist in developing a framework for the use of entomopathogenic nematodes against codling moth within an IPM system.

Keywords: *Cydia pomonella*, *Heterorhabditis zealandica*

Contributed Paper **Wednesday, 17:30** **170**

Evaluation of Pakistani strains of entomopathogenic nematode to some major stored product insect pests

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Eight Pakistani strains of entomopathogenic nematodes belonging to the genus Steinernema and Heterorhabditis were evaluated against different stages of *Lasioderma serricorne*, *Callosobruchus chinensis*, *Sitophilus oryzae* and *Tribolium castaneum*. These nematodes included: *S. pakistanense* Shahina, Anis, Reid and Maqbool (Ham 10 strain); *S. asiaticum* Anis, Shahina, Reid and Rowe (211 strain); *S. abbasi* Elawad, Ahmad and Reid (507 strain); *S. siamkayai* Stock, Somsook and Reid (157 strain); *S. feltiae* Filipjev (A05 strains); *S. carpocapsae* Weiser (T51 strain); *H. bacteriophora* Poinar (1743 strain); *H. indica* Poinar, Karunakar and David (HAM-64 strain). Activity of all strains was determined at four different concentrations in Petri dishes and in concrete arenas. The concrete arenas were used to mimic field applications to cracks or crevices that may be used as refugia by the target pest. A significant dose/concentration effect was detected for all nematode species tested. Among the species, *S. feltiae* (A05 strains) was the least virulent, whereas the highest percentage mortality was observed in *H. bacteriophora* (1743 strain), *S. siamkayai* (157 strain), *S. pakistanense* (Ham 10 strain) and H. indica (HAM-64 strain). Entomopathogenic nematodes have unique host seeking and reproductive strategies and have anatomical characteristics that encourage consideration of their application in stored product pest programmes.

Keywords: Steinernema, Heterorhabditis, stored product insect, entomopathogenic nematode, biological control

Contributed Paper **Wednesday, 17:45** **171**

Current and future prospective of Entomopathogenic nematode in Pakistan

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Entomopathogenic nematode (EPNs) is a new type of Biopesticide which has a great potential for controlling many important agricultural pests. An extensive survey for EPNs was carried out by Shahina and Maqbool in 1996. More than 5000 soil samples were collected from various localities, plantations and soil type. EPNs were isolated from soil sample by baiting with Galleria larvae and identified to species level by the combination of molecular identification (PCR-RFLP and sequence ITS1-5.8S-ITS2 region of rDNA) and morphological means. As a results two new species viz., *Steinernema pakistanense* Shahina *et al*., 2001, *S. asiaticum* Anis *et al*., 2002 were described and six known species *S. abbasi* Elawad *et al*., 1997, *S. siamkayai* Stock *et al*., 1998, *S. feltiae* Filipjev, 1934, *S. carpocapsae*, *Heterorhabditis indica* Poinar

et al., 1992 and *H. bacteriophora* Poinar, 1975, were recovered. Three species of symbiotic bacteria *Photorhabdus luminescence* Boemare *et al*., 1993, *Xenorhabdus nematophila* and *X. bovenii* Akhurst, 1980 have been reported for the first time from Pakistan (Shahina *et al*., 2004).

Keywords: Biopesticide, PCR-RFLP, *Steinernema pakistanense*

Wednesday, 16:30-18:30
VIEW POSTERS
Bacteria, COST: 862, Virus and Fungi
(Authors stand by posters)

Wednesday, 18:30-20:30
Nihat Usta
DINNER AND AUCTION

Wednesday, 20:30-22:30
SIP DIVISION BUSINESS MEETING

Wednesday, 21:00-22:30
COST 862, MC Meeting

Wednesday, 21:00-22:30
Fahri Kuran
Microbial Control
Business Meeting
Workshop: Pathogens of arthropods other than insects
Chair: Surendra Dara

Workshop Wednesday, 21:00 172

Host pathogen interaction between ticks and entomopathogenic fungi

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Ticks are highly important ectoparasites which transmit variable pathogens to both domestic animals and man. Tick control is based mainly on chemical acaricides which are harmful to the environment. Various studies demonstrated the susceptibility of different tick species to entomopathogenic fungi (EPF), particularly *Metarhizium anisopliae*, and suggested their use as biological control agents. In the recent years our team has investigated the potential use of EPF against ticks. This included elucidation of the factors determining EPF pathogenicity and the susceptibility of the target tick. At the early stages of the infection process we observed that adhesion of fungi conidia to different tick species with variable susceptibility to the fungi lacked any specificity. However, the initial pathogenic process (germination, appressorium differentiation) of the fungi conidia to epicuticular extracts from ticks was highly dependent on both the susceptibility level of various tick species and on the fungal strain virulence. We utilized *M. anisopliae* expressing GFP to follow the pathogenic process *in-vivo*. Different pattern of development was observed among susceptible and resistant ticks. Moreover, differences in response to EPF infection was recorded among various tick species which are resistant to the fungi. The implications of the information obtained in our studies on the selection of EPF strains for control of ticks will be discussed.

Keywords: Ticks, Biological control, entomopathogenic fungi

Workshop Wednesday, 21:20 173

Iridovirus and Rickettsiella infections of isopods.

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Infections by iridoviruses (family *Iridoviridae*) and bacteria of the genus *Rickettsiella* were originally described in insects, and isolates of these pathogens have been most commonly described from a wide range of species of the class Insecta. In general, these two types of pathogens have a very broad host range, occurring in insect species of many different orders, more so than most other types of obligate pathogens of insects, such as *Bacillus thuringiensis*, baculoviruses, and fungi. What is generally not known is that iridoviruses, and to some extent *Rickettsiella* species, also cause disease among a diverse range of non-insect hosts. This presentation will focus on the pathology of infections by these pathogens in crustaceans, specifically, in isopods, which are commonly known as woodlice and pillbugs, one of the few types of terrestrial crustaceans. Iridovirus infections in isopods are easily recognized because they impart a deep blue to purple color to infected individuals, caused by the crystallization of virions in epidermal tissues. These infections have been known for centuries, as they were so easily recognized, but were thought to be a type of genetic mutant. In 1980 it was shown in two isopods, *Armadillidium vulgare* and *Porcellio dilatatus*, in California that the blue color was due to an iridovirus. Subsequently, numerous species of isopods worldwide have been shown to be hosts for iridoviruses, though infection rates are low, much less than 1%. The mechanism of infection is not known. However, it is known that most tissues are infected, and that the disease is fatal, with most isopods dying within 10-14 days after infection. Infections by *Rickettsiella* species are chronic, but fatal, and mortality can run as high as 10%. These infections are also easily recognized – they cause internal tissues, especially connective tissue, to become opaque white. As the disease progresses over a period of many weeks, the infected white tissues are easily recognized through the ventral surface with the naked eye. Characteristics of infections by these pathogens in aquatic crustaceans will also be briefly reviewed in this presentation.

Workshop Wednesday, 21:40 174

Pathogens of predatory mites used for biological pest control

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Several species of predatory mites are mass-reared for augmentative biological control in agroecosystems. A variety of pathogens are known to infect the spider mite predators *Metaseiulus occidentalis* and *Phytoseiulus persimilis* and the thrips predator, *Neoseiulus* (*Amblyseius*) *cucumeris*. Viruses and bacteria (*Rickettsiella* and *Wolbachia*) have been reported but their effects are not fully understood.

Poor predator performance is associated with an accumulation of dumbbell-shaped, birefringent crystals within the gut lumen of *P. persimilis*. Crystals are thought to be normal excretory products but excessive accumulation of these crystals within the rectum and Malpighian tubules results in the appearance of white dots or stripes that are visible through the cuticle. In some cases, excess crystal accumulation is associated with the bacterial pathogen, *Acaricomes phytoseiuli*. Affected mites may exhibit permanent changes in foraging behaviour, become emaciated and die. Microsporidia are common pathogens of predatory mites, infecting all three mite species. Infected mites may exhibit significant reductions in fecundity and adult survival, reduced predation capacity, and male-biased sex ratios. Microsporidia-infected mites may not show any obvious symptoms and because pathogen effects are often subtle, microsporidia may remain unnoticed in mass-rearings until colonies fail to thrive. Microsporidia are transmitted both horizontally and vertically. In some cases, these pathogens may be eliminated from mass-rearings by isolating uninfected individuals (Pasteur method) and using them to rear uninfected colonies. Microsporidia have also been reduced in colonies when infected individuals are reared at elevated temperatures. Healthy colonies may then be established from the progeny of heat-treated adults.

Keywords: Predatory mites, microsporidia, bacteria

Workshop Wednesday, 22:00 175

Interactions between a large DNA virus (WSSV) and an invertebrate host (shrimp)

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White spot syndrome virus (WSSV), is the causative agent of a disease that has led to severe mortalities of cultured shrimps globally. Here we review some of the ways in which WSSV genes interact with the host cell machinery. (1) Against the host defense of apoptosis, WSSV deploys at least two viral proteins that prevent apoptosis in target cells. (2) It is already well known that WSSV is able to suppress RNAi, and by using an RNAi suppression assay system, we have now identified four WSSV gene products as potential RNA silencing suppressors. (3) We discovered and confirmed that IRES-mediated translation is widely used by WSSV late genes. This allows viral proteins to be expressed even as the host cell translation machinery is starting to break down. (4) The WSSV nonstructural protein ICP11 is a recently-discovered DNA mimic protein that strips histone from the nucleosome, acts as a nuclease enhancer, and disrupts chromosome structure. (5) At high water temperature (32-33℃), WSSV's replication is suppressed. Proteomic and knockdown studies suggest that HSP70 and ALDH are both likely to be key factors that act to inhibit WSSV replication under hyperthermic conditions. (6) Although activated STAT is part of a cell-defense signaling pathway it is co-opted by the promoter of the viral *ie1* gene and used to enhance *ie1* expression and viral replication. Since STAT is also activated in response to environmental and physiological stressors, this explains why these stressors can trigger WSSV replication.

Keywords: WSSV, Shrimp, host-virus interactions

Wednesday, 21:00-22:30
Nihat Turan 2

Diseases of Benefical Invertebrates
Business Meeting
Workshop: Histopathology of beneficial invertebrates
Chair: Grant Stentiford

Workshop Wednesday, 21:00 176

Histopathology of crustaceans: a frontline tool in pathogen discovery and diagnosis

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Wild captured and farmed crustaceans form a significant proportion of global aquatic food production. In 2006, total crustacean production from these two sectors exceeded 10 million metric tonnes with a first sale value of almost $40bn. Disease is a major bottle-neck to production in aquaculture (e.g. white spot disease in farmed penaeids) while in the capture sector, mortality due to pathogens is often unrecorded and therefore, largely misunderstood. Despite the increasing commercial importance of crustaceans, we report on the relative dearth of infortmation concerning significant diseases of this group and furthermore, the lack of global expertise in the detection and diagnosis of known and emergent pathogens. Several factors, including the absence of continuous cell lines for viral propagation appear to have contributed to this defecit. Furthermore, a movement away from 'traditional' approaches to disease investigation (e.g. based upon histology and electron microscopy) to a solely molecular approach have the potential to limit detection to known taxa and further, can often not distinguish between true susceptibility and carrier/vector status of the host. Here, we demonstrate the role of histopathology and electron microscopy as a frontline tool for pathogen discovery and identification in crustacean hosts and provide a framework for studying pathogens and disease in this commercially and ecologically important host group. Furthermore, we describe how these traditional approaches to pathogen diagnosis must sit alongside molecular diagnostic tools when attempting to discriminate mechanical vector, infection and disease status.

Keywords: Histopathology, crustaceans, workshop

Workshop Wednesday, 21:20 177

Histopathology of bivalve molluscs

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Bivalve molluscs are an important component of aquatic ecosystems, and are an important food source

for a number of predators, including Man. Bivalves have been both farmed and fished by humans for several thousand years, being used as a food source and, in some cases, as decorative pieces. Furthermore, bivalves have been used as ecosystem health indicators since they can bioaccumulate selected contaminants through the filtration of large volumes of water. In addition, they are hosts to a wide range of pathogens and are integral in the lifecycles of parasites such as digeneans, acting as primary and secondary hosts for these pathogens. Furthermore, they act as hosts for pathogens such as viruses, bacteria, protistans, metazoans as well as a wide range of commensals and symbionts. Since bivalves, in common with other invertebrates, have somewhat primitive defence mechanisms against pathogens, there are limited options available to the host for destroying pathogens. This talk will consider the main defences mechanisms used by bivalves to cope with infectious agents. In addition, examples of different pathologies normally encountered in bivalves will be shown with a guide to the main diagnostic features used to identify these pathogens and pathologies.

Keywords: Parasite; disease; pathology; immune response

Workshop Wednesday, 21:40 178

Histopathology of bees: A neglected discipline

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Despite the importance of honey bees as commercial pollinators and the current problems with honey bee decline which is thought to be mainly caused by pathogens, honey bee pathology is still in its infancy. Our knowledge and understanding of honey bee pathogens is fragmentary and molecular data do exist only for a few pathogens. Likewise, little is known about host-pathogen interactions at the individual insect level and less even at the cellular level. Hence, it is not surprising that also the field of honey bee histopathology is a neglected discipline. We here present what is known in this field, but also what is lacking and what is urgently needed.

Keywords: Histopathology, honey bees

Workshop Wednesday, 22:00 179

A dyeing art: Histopathology for the assessment of health and disease in terrestrial insect populations

Gemma Baron[1]; Grant Stentiford[2]; Matthew Green[3]; Ruth Hicks[3]; Helen Roy[4]; Helen Hesketh[4]; Gabriele Rondoni[5]; Morag McCracken[4]; Rosemary Hails[4]
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Insect pathogens have been widely described in agricultural systems, but are rarely recorded from wild non-pest species. Contributing reasons for this include the ephemeral nature of overtly infected insects, coupled with a lack of techniques available to detect chronic infections in insects. A recent collaboration formed through the Society for Invertebrate Pathology has lead to the application of field sampling and histopathological techniques, currently used to detect pathogens in populations of marine crustaceans, to populations of non-pest terrestrial insects.
Two model species were chosen for this study, the adonis blue butterfly *Polyommatus bellargus* (Lepidoptera: Lycaenidae), a chalk grassland species native to the UK and Western Europe, and the harlequin ladybird *Harmonia axyridis* (Coleoptera: Coccinellidae), an invasive ladybird, introduced to Europe and North America. These species were sampled from selected sites in Southern England. Specimens were fixed with a range of fixatives, and processed for histological examination. Stained histological sections from individual specimens were examined to gain an insight into normal anatomy and histology in addition to the presence of pathology and pathogenic agents.
This study has demonstrated the potential of using histopathological techniques for studying health and disease in populations of terrestrial insects. The re-emergence of such 'traditional' approaches to insect health assessment alongside molecular diagnostics for specific known pests provides unique opportunities to study disease susceptibility and pathogenesis in a variety of natural terrestrial systems.
During this talk we will present images of histological sections from this research, and discuss the implications of using such techniques for the study of beneficial and invasive insect species.

Keywords: Histopathological, terrestrial invertebrates, *Harmonia axyridis*, *Polyommatus bellargus*

20:45-21:45
Demonstration Room

Student and Post Docs CommiteeMeeting

Workshop Wednesday, 21:00 180

How to prepare for an Interview.

S. Patricia Stock
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Job interviews, no matter what type of position one is interviewing for can be a stressful process, especially for first-timers. Graduate students, recent graduates, and postdocs will have to face this process a few times, before they find their suitable mentor, lab or "dream job". Usually job interviews for academic positions include interviews with faculty members, students and a number of campus representatives. In addition to , the candidate will be require to give one or two formal presentations, and participate in social events that are usually planned to get to know them betterl. So, how can you prepare for the academic job interview for a faculty position at a college or university? One critical issue is to be prepared for quite a diverse range of questions and be ready to answer them. In this presentation I will summarize my personal experience in interviewing for an academic position from both the interviewee and the interviewer perspective.

Workshop Wednesday, 21:00 181

Co-operation and collaboration (Liasons) in thematic research

Jorgen Eilenberg
Copenhagen University, Copenhagen, DK

22:45 **Buses depart for hotels**

POSTERS
Bacteria, COST: 862, Virus and Fungi

BACTERIA

Poster / Bacteria Wednesday, 16:30 B-01

Study of the bacterial flora as a biological control agent of *Lymantor coryli* Perris (Col.:Curculionidae)

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Hazelnut is one of the most important products in Blacksea Region of Turkey for both production and exportion. According to the recent observations, the damage of bark beetles (Col.:Curculionidae) are gradually increasing specially *Lymantor coryli* (Perris) are the most common species.The aim of this study is to find a more effective and safe biological control agent against *Lymantor coryli.* We investigated the bacterial flora of the pest that were collected from the vicinity of Giresun, TURKEY. We isolated and identified bacteria from the *Lymantor coryli* and determined. Based on morphological, physiological (pH, NaCl, temperature) and biochemical tests (VITEK 2 and MIS) , bacterial flora were identifed as *Rahnella aqualitis* (Lc1), *Acinetobacter baumannii* (Lc2), *Sphingobacterium multivorum* (Lc3), Serratia sp. (Lc4), *Aeromonas hydrophila* (Lc5), *Hafnia alvei* (Lc6), *Enterobacteriaceae* (Lc7 and Lc9), *Acinetobacter* sp (Lc8), *Pseudomonas* sp. (Lc10), *Pantoea agglomerans* (Lc11), *Serratia marcescens* (Lc12), *Pseudomonas fluorescens* (Lc13), *Pseudomonas putida* (Lc14).
Future studies will be tested insectisidal activity against *Lymantor coryli* and other hazelnut pests.

Keywords: Hazelnut, *Lymantor coryli*, biological control, bacterial flora

Poster / Bacteria Wednesday, 16:30 B-02

The first study on the bacterial flora of the Xyleborus xylographus Say (Coleoptera: Curculionidae)

Ahmet Katı[1]; **Serpil Ugras**[2]; **Huseyin Yılmaz**[2]; **Hatice Katı**[1]
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The branch breaking beetle, *Xyleborus xylographus* Say (Coleoptera: Curculionidae), is one of the most harmful insect pest in the hazelnut fields in Turkey. In this study, we investigated the bacterial flora of *X. xylographus* collected from the different populations in the hazelnut fields of the Eastern Black Sea Region of Turkey in 2008. Sixteen different bacteria were isolated from healthy, diseased and dead specimens based on the color of colony and morphology. According to morphological, physiological and biochemical properties with VITEK Identification System and total cellular fatty acid profile by Microbial Identification System (MIS). Isolates were identified as *Acinetobacter haemolyticus* (Xx1), *Acinetobacter* sp. (Xx2) *Stenotrophomonas maltophilia* (Xx3), *Pseudomonas fluorescens* (Xx4), *Staphylococcus sciuri* (Xx5), *Staphylococcus warneri* (Xx6), *Enterobacteriaceae* (Xx7, Xx11), *Staphylococcus hominis* (Xx8), *Pseudomonas* sp. (Xx9), *Erwinia sp.* (Xx10), *Brevibacterium casei* (Xx12), *Pantoea agglomerans* (Xx13), *Pseudomonas* sp. (Xx14), *Serratia* sp. (Xx15), and *Brevibactecterium epidermidis* (Xx16). This is the first study on the bacterial flora of *X. xylographus.* Further research will be directed to determine the insecticidal effects of these bacterial isolates against *X. xylographus* and other hazelnut pests.

Keywords: *Xyleborus xylographus*, Hazelnut, Bacterial Flora

Poster / Bacteria Wednesday, 16:30 B-03

Crystal structures of Cry34Ab1 and Cry35Ab1 proteins at 2.15 Å resolution

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Cry34Ab1 and Cry35Ab1 are binary insecticidal protein partners active against western corn rootworm (*Diabrotica virgifera virgifera* LeConte) larvae. Cry34/35Ab1 is membrane active and disrupts WCRW midgut epithelial tissue (1, 2). Both crystal structures were solved through a combination of multiple isomorphous replacement (MIR), single isomorphous replacement (SIR) or single anomalous diffraction (SAD). The Cry35Ab1 structure has an elongated rectangular shape. It has two distinct domains with three antiparallel β -sheets forming a barrel-like structure in the N-terminal domain and six helices and three antiparallel β -sheets in the C-terminal domain. The Cry34Ab1 structure has only one distinct structural domain containing 117 amino acids. The protein folds into a typical β-sandwich conformation; the entire β -sandwich has a relatively flat, layer-like conformation and two slightly twisted β -sheets. The protein crystal structures of Cry34Ab1 and Cry35Ab1 are distinct from one another, and from the three-domain coleopteran-active Bt Cry proteins including Cry3Bb1. Protein structure differences are one line of information supporting the combined use of Cry34/35Ab1 and Cry3Bb1 as a strategy for WCRW resistance management.

Keywords: *Bacillus, thuringiensis*, Cry34Ab1, Cry35Ab1, Diabrotica

Poster / Bacteria **Wednesday, 16:30 B-04**

Cysteine substitution in Cry7Ba1 crystal protein from *Bacillus thuringiensis* improves the crystal solubility and recovers its toxicity to *Plutella xylostella* larvae

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Bacillus thuringiensis produces insecticidal crystals during its sporulation, which can be dissolved in the alkaline midgut of insects, and then exert their toxicity. It was found the crystals of *B. thuringiensis* subsp. *huazhonggiensis* strain YBT-978 (serotype H_{40}) cannot be dissolved at pH9.5 and did not show toxicity to Lepidoptera larvae. However, this kind of crystals can be dissolved at pH12.5, and the solubilized crystal protein exhibit high toxicity to *P. xylostella* larvae. When the crystal protein encoding gene *cry7Ba1* was expressed in acrystalliferous strain BMB171, the resulting Cry7Ba1 crystals cannot be dissolved at low pH (9.5) and have no insecticidal activity, while can be dissolved at pH12.5 and the solubilized crystal protein keeps toxicity. After the C-terminal half of Cry7Ba1 protein was replaced by that of Cry1Ac and Cry1C, the recombinant crystal proteins could be dissolved at pH 9.5, and exhibited toxicity. It was found there are 7 cysteines located in Cry7Ba1 C-terminal half, which amount is more than that in Cry1 proteins. Six of cysteine mutations (C834S, C854S, C697S, C1059S, C1101S, and C1154S) were constructed by substituting with serine. Crystals from three mutations (C834S, C854S and C697S) can be dissolved at lower pH (9.5-10.5). What' more, mutation C834S recovers the toxicity against *P. xylostella* larvae. These findings suggest a single cysteine substitution in C-terminal half of crystal protein has a major practical significance with respect to recover the solubility and toxicity of some nontoxic crystals.

Keywords: *Bacillus thuringiensis*, crystal solubilization, cysteine substitution

Poster / Bacteria **Wednesday, 16:30 B-05**

Screening and genetic characterization of *Bacillus thuringiensis* strains for the control of *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae)

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Aedes aegypti is the major vector of dengue virus and urban yellow fever and represents a significant public health problem in Brazil. The rapid increase in mosquito resistance to various chemical insecticides has resulted in the increase in research of biological products as those based in *Bacillus thuringiensis* var. *israelensis* (Bti). However, in Brazil, the use of Bti products is still incipient. The most of these products are imported, causing corresponding increases in costs, and leading to a growing need for developing technologies that can generate Bti-based products to insect control programs. In this work, a total of 28 B. thuringiensis strains were characterized by PCR analysis using primers for *cry* and *cyt* genes encoding proteins active against mosquitoes (Cry4A, Cry4B, Cry10, Cry11, Cyt1 and Cyt2) and were submitted to selective bioassays against third instar *Aedes aegypti* larvae. About 33% of the strains contained all the studied genes and about 37% of the strains showed high toxicity activity against *A. aegypti* larvae. Ten strains were selected for bioassays in different temperatures and pH values, and for one strain (*B. thuringiensis* BR14), the obtained LC50 values were statistically lower than for *B. thuringiensis* var. *israelensis* IPS-82. Others *cry* genes of this strain will be investigated and characterized, and the culture conditions optimized, aiming the development of a new B. thuringiensis product for the *A. aegypti* control.

Keywords: CAPES, CNPq, Brazil

Poster / Bacteria **Wednesday, 16:30 B-06**

Screening of *Bacillus thuringiensis* strains against economically important insect pests in Brazil

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In Brazil, synthetic insecticides have been effectively used, but their use has become problematic because of a multiplicity of factors, causing an increased interest in the use of *Bacillus thuringiensis* (Bt) products. However, in Brazil, most of these products must be imported, causing corresponding increases in costs, and leading to a growing need for developing technologies that can generate products based on *B. thuringiensis* in Brazil to be used in insect control programs. A total of 70 Bt strains were submitted to selective bioassays against four economically important insects; the lepidopterans Pseudoplusia includens, *Chlosyne lacinia saundersii*, and *Spodoptera frugiperda*, and the coleopteran *Alphitobius diaperinus*. In addition, Bt strains were characterized by the presence of the *cry*1, *cry*2 and *cry*3 genes by PCR analysis and for the presence of crystal proteins by SDS-PAGE. B. thuringiensis strains that exhibited highly toxic activity were selected for the toxicity bioassays. Most of the Bt strains reacted with the *cry*1 and *cry*2 primers; but only a few strains reacted with *cry*3 primers. This screening identified new *B. thuringiensis* strains that showed high activity against the three lepidopteran species, which can be available in experimental fields, and subsequently used in biopesticide formulation, as well as their *cry* genes will be characterized and used in Bt crops. These strategies can be used in biological

control applied to the Integrated Pest Management of several cultures economically important in Brazil. None of the tested strains presented activity against *A. diaperinus*.

Keywords: CNPq, CAPES, Brazil

Poster / Bacteria **Wednesday, 16:30 B-07**

Expression of Cry1AbMod toxin in transgenic tobacco and its effectiveness to control *Manduca sexta* larvae

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Bacillus thuringiensis produce crystal proteins with high toxicity against insect pests of many commercial crops. Cry genes encoding lepidopteran-specific insecticidal proteins have been cloned and expressed in transgenic plants since 1996, resulting in insect resistant plants. The advantages of using Bt-crops include the following: reduce use of chemical insecticide for pest control; highly effective pest control; and increased crop yields. However, insect resistance to Cry toxins is the main threat for the continue use of this technology. Recently, engineered modified Bt-toxins lacking α-helix 1 (Cry1AMod), had been proposed as novel strategy to avoid insect resistance to Bt-crops. In this work, we design a Cry1AbMod toxin with a preferential plant codon usage and demonstrate its expression in tobacco transgenic plants. Our results show that Cry1AbMod protein accumulated in leaf tissue and show toxicity to *M. sexta* larvae as demonstrated by bioassays using contaminated diet with total protein from Bt-tobacco. Our result shows that Cry1AMod toxins can be efficiently express in plants and therefore could useful to control insect resistance to Bt toxins in the future.

Keywords: Cry toxin trasgenic plant

Poster / Bacteria **Wednesday, 16:30 B-08**

Head-to-tail screening: an efficient approach to determine the activity–correlative regions of Cry proteins

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Crystal proteins, produced by *Bacillus thuringiensis* (Bt), is widely used in agriculture all over the world due to its specific insecticidal activity and harmless to human and animals. Insecticidal properties draw more and more attention and insecticidal specificity or activity become hot point in this field. Normally, site-directed mutagenesis and domain exchange are used to explore key amino acids or regions for specificity or activity. Some achievements were reached, such as some amino acids in loops are responsible for activity and domain is one of specificity determinants. However, the study of insecticidal specificity or activity is far away certainty; the race for key amino acids or regions is still on.

Here, we present a strategy of head-to-tail screening to identify the activity–correlative regions (ACR) of proteins by comparing the activities of the parent genes with those of the recombinants of a random and single-crossover recombination library (RSCRL) derived from 2 homologous parent genes with different activities. We developed a novel in vitro recombination method based on dideoxy random termination and re-extension with different templates to produce the RSCRL for screening. This method required several PCR procedures with DNA polymerases with different 3′→5′ exonuclease activity such as *KOD* DNA polymerase and *Taq* DNA polymerase. Using this method, we constructed an RSCRL of two Bt delta-endotoxin genes, *cry2Aa* and *cry2Ad*, which share 89.57% identity in DNA sequence and 86.10% identity in their encoded amino acid sequence. Subsequently, we performed bioassay and SWISS-MODEL analysis to identify 3 ACRs of Cry2Aa, which mediated toxicity to *Ostrinia furnacalis*, *Plutella xylostella*, and *Chilo suppressalis*, respectively. For *Ostrinia furnacalis*, the ACR is located in the region of 403S to 415S. For *Plutella xylostella*, the ACR is located in the region of 421Y to 439T. For *Chilo suppressalis*, the ACR is located in the region of 440R to 454S. The successful use of this novel method laid the foundation for further study, as well as broadened the channels for the study of relationship between protein structure and function. Furthermore, two Bt proteins with high insecticidal activity could be recombined by this method, to chimerize a new proteins with much higher insecticidal activity.

Keywords: Cry2 crystal proteins, gene recombination, key regions, structure, function

Poster / Bacteria **Wednesday, 16:30 B-09**

Evaluation of 2nd generation of *Bt*-transgenic sugar beet lines *vs.* their three lepidopteran pests

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The 2nd generation lines (T_1) derivated from 9 transgenic sugar beet lines (T_0), containing a synthetic *cry1Ab* gene of *Bacillus thuringiensis* have been evaluated as controlling measures of three lepidopteran pests of sugar beet, *Beta vulgaris* L., such as Black/common cutworm *Agrotis segetum* (Denis and Schiffermuller), Egyption cotton leafworm *Spodoptera littoralis* (Boisduval) and sugarbeet armyworm *S. exigua* (Huebner) (Lep.: Novtuidae). In current study in CRD experiments, with four replications, 40 neonate larvae (10 larvae per replication) were feed on the leaves of the sugar beet transgenic lines and 2 non-transgenic lines in Petri dish at *in vitro* conditions. Larval mortality, larval weight loss (anti-feeding effects of the leaves) and damage index (loss weight of the leaves) of the pests were recorded after 3 and 6 days. After normality tests of data and correction according to Abbot's formula, the ANOVA results showed the significantly

difference among lines in most of the evaluated factors. The evaluation experiments by *S. littoralis* and *S. exigua* screened the lines and showed after 3 days, up to 25% mortality and after 6 days, up to 45%. Meanwhile, for *A. segetum* after 6 days, less than 20% mortality has been recorded. A few transgenic lines such as $S_{36\text{-}13}$, the most effective line among evaluated transgenic lines, have been selected for further analyses and investigations.

Keywords: Bacillus thuringiensis, cry1Ab gene, Lepidopteran pests, Spodoptera littoralis, S. exigua, Agrotis segetum, Transgenic Sugar beet

Poster / Bacteria **Wednesday, 16:30 B-10**

In vivo virulence of *Bacillus cereus* and *Bacillus weihenstephanensis* at different temperatures in *Galleria mellonella* larvae

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The entmopathogen *Bacillus thuringiensis* (Bt) is closely related to *Bacillus cereus* (Bc) which is an opportunistic human pathogen, recognised as a cause of food borne disease. Food borne pathogenicity is linked to the production of toxins. The closely related *Bacillus weihenstephanensis* (Bw) is a cold-adapted species which, until 1998, was not taxonomically distinguished from Bc. It is not known whether Bw can cause food borne or other forms of diseases. Investigations on Bw strains have shown presence of the genes encoding foodborne disease factors in Bc (Stenfors et al., 2002). Cytotoxic activity of supernatants from cultures grown at temperatures from 12 °C to 37 °C showed that Bw strains conferred higher cytotoxicity at low temperatures. The present study compares virulence of Bw and Bc strains in an insect model (*Galleria mellonella*) already used for Bt and Bc infections (Fedhila et al., 2006, 2010). Virulence was investigated via two routes (injection into the hemocoel, oral force feeding) at two temperatures (15 °C, 37 °C). Larval mortality was scored over time. Temperature dependent species differences in virulence were observed between Bc and Bw. At 15 °C both species elicited a high level of virulence, while at 37 °C, virulence of Bw was negligible and of Bc moderate to high. Details of these results as well as the kinetics of mortality induction, differences between route of infection, and strain differences within between species are discussed. Our results suggest that Bw, Bc and *B. thuringiensis* strains may share common ecological niches including invertebrates living in temperate climate.

Keywords: *Bacillsus cereus*, *Bacillus weihenstephanensis*, *Galleria mellonella*, virulence, cytotoxicity

Poster / Bacteria **Wednesday, 16:30 B-11**

In vitro assay of alternative phytosanitary Products on *Bacillus thuringiensis* var. *kurstaki*

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Alternative phytosanitary products (APP) and *Bacillus thuringiensis* var. *kurstaki* (Btk) are important tactics for pests control on agroecological production systems. Although many of APP are safer than the conventionalphytosanitary products, there are few information about interactions of APP and entomopathogens. Thus, this work aimed to evaluate the effect of alternative (APP) on the spore germination and the crystal toxicity of Btk. So eight commercial products were evaluated in the recommended (RC), half (½RC) and double concentration (2RC). On test 1, the products where mixed with sterilized distilled water and nutrient broth (NB), and then inoculated with Btk. The evaluations were made by quantifying the CFU 14 and 18h after inoculation. On the test 2, the mixture Btk+APP was added on the surface of the artificial diet for *Anticarsia gemmatalis*, and the mortality was evaluated after 12, 24, 48 and 72h. On the test 1, Mattan Plus® was the only product that did not affect the spore germination, both in ½RC and RC when mixed with distilled water, and in the three concentrations when mixed with NB. Both Stubble-Aid® when mixed with water, and Agro-Mos® when mixed with NB, proportionally reduced the germination when the concentration was increased. On test 2, the Bordeaux Mixture in all concentration caused alterations on the Btk, Biogermex® on the RC+Btk and 2RC+Btk, and Agro-Mos® on the RC+Btk, showed partial negative effects on the crystal toxicity, reducing the mortality with 48h.

Keywords: Entomopathogenic bacteria, compatibility, IPM

Poster / Bacteria **Wednesday, 16:30 B-12**

Biochemical and molecular characterization of delta-endotoxins in *Bacillus thuringiensis* native strain

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Agriculture is one of the bases of Argentina's economy. Some lepidopteran and coleopteran species cause significant damage to many agricultural important crops. *Anticarsia gemmatalis*, *Spodoptera frugiperda*, *Diabrotica speciosa* and *Anthonomus grandis* are currently endangering large areas of soybean, wheat, corn, rice, cotton and forage grasses. We described a *Bacillus thuringiensis* strain (FCC7) isolated from a soil sample in Argentina. This strain was shown to be toxic against larvae of lepidopterans (*Anticarsia gemmatalis* and *Spodoptera frugiperda*) and coleopterans (*Diabrotica speciosa*, *Tenebrio molitor* and *Anthonomus grandis*). It has a rounded crystal protein inclusion and its SDS-PAGE polypeptide pattern showed the presence of two major bands of 50 and 135 kDa. Through an approach based on PCR amplification of DNA fragments with degenerated oligonucleotides, we could retrieve partial

cry homologous sequences, and a TAIL-PCR methodology allowed us to obtain a full length *cry* sequence, after confirmed by primer walking. BLASTp analysis using the deduced amino-acid sequence indicated that this novel sequence is related to proteins of the Cry8Aa type. In order to obtain other coding sequences from FCC7 strain, we used a PCR-DGGE based approach.We found two partial sequences homologous to *cry1I* genes. These results indicate that a combination of at least two Cry proteins may be responsible for FCC7 toxicity.
Suported by ANPCyT (PICT-2007-02069) and Universidad Nacional de Mar del Plata Proyecto (15E/329 EXA 382/07).

Keywords: *Bacillus thuringiensis*, Cry8 type gene, lepidopterans and coleopterans pests

Poster / Bacteria **Wednesday, 16:30 B-13**

Yersinia entomophaga, a potential new biopesticide for locusts

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Yersinia entomophaga is an entomopathogenic bacterium isolated from the New Zealand grass grub *Costelytra zealandica* (Coleoptera: Scarabaeidae). Host range testing has found the bacterium to infect a number of Coleoptera and Lepidoptera species, along with the Othopteran *Locusta migratoria* L. After ingestion of *Y. entomophaga* MH96, death typically occurs within 2-5 day post infection. *Yersinia entomophaga* MH96 secretes large amounts of an insecticidal toxin complex, termed super toxin complex (STc), which is also active to *L. migratoria*. The potential of *Y. entomophaga* and its STc to kill *L. migratoria* L, was assessed in a series of laboratory studies under temperatures of either 24 or 37 °C. Locusts (1-3rd instar) were allowed to feed on ryegrass (*Lolium multiflorum*) sprayed with either *Y. entomophaga;* a semi purified STc and a *Y. entomophaga* STc deletion derivative unable to produce the STc (Stc deletion variant). Results showed that against early instar locusts, mortality commenced within 24 hrs of exposure to *Y. entomophaga*, with generally 100% mortality after 72 h. Ingestion of semi purified STc caused mortality in 1st instar locusts of 34-97% after 6 d, with the temperature locusts held at influencing the level of mortality. Exposure to 'STc deletion variant' caused minimal mortality (12%) after 6 d compared to STc, indicating that the STc is the main virulence determinant of *Y. entomophaga*.
Both *Y. entomophaga* and the STc are able to kill *L. migratoria*. Activity against other pest locust or grasshopper species has yet to be assessed, but this research has indicated the potential of a new biopesticide targeting Acrididae.

Keywords: Locust, migratory locust, biopesticides, locust control

Poster / Bacteria **Wednesday, 16:30 B-14**

Cloning, characterisation and expression of *chitinase* A, B and C Genes isolated from *Serratia marcescens* originating from *Helicoverpa armigera* and determining their insecticidal activity

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There are significant amount of chitin in the structure of peritrophic membrane which surround cuticle and gut in insects. Degradation of the chitin found in the structure of cuticle and peritrophic membrane by the chitinase enzymes, decreases the feeding and the defence of the insect and thus makes the insect weak. Therefore chitinases are important enzymes which have potential as being use biological control agent against harmful insects.
Chitinases form an intensive dispersion among organisms. These include bacteria, fungi, higher plants, insects, crustacean and some vertebrates.
In this study, a *Serratia marcescens* bacterium was isolated from naturaly dead *Helicoverpa armigera* larvae. The genes encoding *chitinase* A (*chi*A), B (*chi*B) and C (*chi*C) were amplified from this bacteria by PCR using dejenerate primers. For identifying the nucleotide sequence of the amplified genes, their sequence analyses were done. Sequence results showed that , *chitinase* A and B have 99 and 97 % homologies, respectively with the *Serretia marcescens* (BJL200) from literature, and *chitinase* C has 96% homology with the *Serretia marcescens* chiC1 from literature. The sequenced chitinase genes were cloned to the pET-28a(+) expression vector and their proteins were expressed in *Escherichia coli*. These expressed proteins were purified and their SDS-PAGE analyses were performed. As a result, protein bands for *chitinase* A, B and C genes were detected on polyacrylamide gel at 57, 53, and 50 kDa sizes, respectively.
Insecticidal activities of these proteins were tested on *Malacosoma neustria* 3rd instar larvae. Test results showed that, chitinase A, B and C have 47, 50 and 66 % insecticidal activities on *Malacosoma neustria* larvae, respectively.

Keywords: *Serratia marcescens*, ChiA, ChiB, ChiC, Chitinase, Insecticidal activity

Poster / Bacteria **Wednesday, 16:30 B-15**

Effect of the symbiotic bacterium, *Xenorhabdus indica,* from *Steinernema abbasi* Taiwan strain on some microorganisms and insect cell lines

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The entomopathogenic nematode, *Steinernema abbasi* Taiwan strain, is a species isolated from subtropical region. Its symbiotic bacterium, *Xenorhabdus indica*, was found to be strongly inhibitory to the bacterium, *Vibrio parahaemolyticus* CCRC10806, whereas was less inhibitory to other bacteria, e.g., *Staphylococcus aureus* CCRC12652, *Klebsiella pneumoniae* CCRC10694, and *Escherichia coli* JM109, and a plant pathogenic fungus, *Botrytis cinerea*. In addition, the cultured filtrates of the primary form of *X. indica* were toxic to *SF*21 and S2 cell lines, causing cellular necrosis even after being autoclaved for 20 min, while those of the secondary form were not toxic to both cell lines. However, the cultured filtrates of both forms

were not toxic to a mammalian cell line, BHK21. In *in vivo* assays, the viable but not dead bacteria could cause hemocytic necrosis in *Spodoptera litura* and *Galleraia mellonlla* larvae. But, the dead bacteria were only capable of causing necrosis and eventually kill hemocytes of *G. mellonella* larvae. In conclusion, the symbiotic bacterium, *X. indica*, is inhibitory to some bacteria and a fungus, and is capable of producing toxic components which could cause hemocytic necrosis in its insect hosts.

Keywords: symbiotic bacterium, entomopathogenic nematode, Xenorhabdus indica, Steinernema abbasi

Poster / Bacteria **Wednesday, 16:30 B-16**

Effect of *Bacillus thuriengiensis* against *Lobesia botrana* Denis and Schiffermuller (Lepidoptera:Tortricidae) in Malekan Region

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2009, the study of effect of *Bacillus thuriengiensis* against grapevine moth, *Lobesia botrana* Denis and Schiffermuller, was carried out in Malekan (located at East Azarbaijan province in Iran).The pest has three generations in the region and *Bt* was used against all generation in the field. The results showed the high control of the pathogen.

Keywords: *Bacillus thuringiensis*, *Lobesia botrana*, Malekan, East Azarbaijan

Poster / Bacteria **Wednesday, 16:30 B-17**

A cricket spiroplasma disease found in *Teleogryllus occipitalis* (Orthoptera: Gryllidae)

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A new *Spiroplasma* sp. was found in the cricket, *Teleogryllus occipitalis*. This spiroplasma causes a chronic disease, the infected crickets are sluggish with an obviously swollen abdomen and die usually within two weeks. The infected crickets were bacteremia, their hemolymph became turbid and showed opaque and milky white. Two bacteria, *Bacillus* sp. and *Spiroplasma* sp., were found in the milky white hemolymph, the latter was considered the main pathogen of this cricket disease. This pathogen was isolated and the SSUrRNA, LSUrRNA, and spiralin genes were cloned and sequenced. Based on each of SSUrRNA and LSUrRNA sequences, this pathogen is closely related to the species of *Spiroplasma platyhelix* from the Dragonfly *Pachydiplax longipennis* in phylogenetic analysis. We proposed that this is a new *Spiroplasma* species and named *Spiroplasma teleogryllusa*.

Keywords: Spiroplasma teleogryllusa, 16s rRna, 23s rRna, cricket

Poster / Bacteria **Wednesday, 16:30 B-18**

Biodegradation of cypermethrin by a newly isolated Actinomycetes HU-S-01 from wastewater sludge

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A new cypermethrin degrading strain was isolated from wastewater sludge using enrichment technique. On the basis of morphological, physiological, biochemical characteristics tests and 16S rDNA sequence analysis, the strain was determined to be a *Streptomyces* species, probably a strain of *Streptomyces parvulus*, so it was designated as *Streptomyces sp* HU-S-01. The strain *Streptomyces sp* HU-S-01 is aerobic and optimum growth temperature for the strain was found to be 26-28, preferential initial pH did range from 6.0-9.0 with the optimum pH 7.5. It was able to tolerate and degrade cypermethrin up to a concentration of 250 mg L^{-1}while the concentration above 300 mg L^{-1} was inhibitory to its growth. This strain can also completely degrade 3-phenoxybenzoic acid within 96 h at the concentration 50mg L^{-1}. The kinetic constants Vmax, Km, Kcat and Kcat/ Km of enzyme for cypermethrin were 1.236 μmol.min^{-1}, 6.418 μmol.mL^{-1}, 13.493 min^{-1}, 2.102 $mL.mol^{-1}$. min^{-1} respectively. The metabolites of cypermethrin were safe for mammals. Biodegradation ability of strain *Streptomyces sp* HU-S-01 without toxic byproducts make it worth for further study as a potential biological agent, for the remediation of soil, water or crops, contaminated with cypermethrin.

Keywords: Biodegradation, *Streptomyces* sp HU-S-01, Cypermethrin, Pyrethroid insecticides, 3-phenoxybenzoic acid

Poster / Bacteria **Wednesday, 16:30 B-19**

Histopathology of midgut in *Bacillus thuringiensis*-susceptible and resistant populations of Diamond Back Moth, *Plutella xylostella* (Lepidoptera: Plutellidae)

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Changes in the midgut structure of two populations of Diamondback moth (DBM), *Plutella xylostella* L. (Lepidoptera: Plutellidae) was studied following three hours after feeding on canola leaves treated with 563 cells/ml of *Bacillus thuriengiensis* concentration. The histopathologic evaluations were done with larvae of both susceptible and resistant populations in time of 3, 12 and 24 hours after treatment. After the fixation, in Bouin for 24 hours, the tissues were dehydrated using ethanol solutions, butanol and xylene. Histological samples were prepared as 4μm thickness cuts and Hematoxylin-eosin method was used to stain the

tissues. Our observations by optical microscopy indicated that disarrangement of epithelial cells, start to lysing and blebbing of their microvilli could be seen in susceptible population midgut after even three hours whereas normal midgut was seen in larvae of resistant population. In 12 hours treatment, thicker epithelial layer was observed in susceptible larvae compared to that of resistant larvae. Completely lysis of microvilli, enlargement of goblet cells, shrink of peritrophic membrane and elongation of columnar cells are most important events in midgut of susceptible larvae in 24 hours treatment. There was no obvious change in midgut of resistant larvae at this time treatment.

Keywords: Histopathology, midgut, *Plutella xylostella*, susceptible, resistant

Poster / Bacteria **Wednesday, 16:30 B-20**

Natural Infection of *Agriotes* sp. with *Rickettsiella* bacteria

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Within the framework of diagnostic investigations of diseased and dead wireworms, *Agriotes* spp., infection with *Rickettsiella* bacteria was for the first time detected using both light and electron microscopy. A phylogenetic analysis based on a 1357bp comprising partial sequence of the gene encoding the small subunit ribosomal RNA (16S rRNA) that had been PCR amplified from insect tissue samples, unambiguously assigned the corresponding bacterium to the genus *Rickettsiella*, order *Legionellales*, of the gamma-Proteobacteria. With respect to species delineation, the internal structure of this genus that aside three currently recognized species comprises numerous non-synonymized pathotypes, is still highly problematic. However, a pronounced homology (>99% sequence identity) of the amplified DNA to the orthologous sequences from the pathotypes `*Rickettsiella melolonthae´* and `*Rickettsiella tipulae´* both of which are currently described as synonyms of the species *Rickettsiella popilliae*, is at least consistent with a possible assignment of the wireworm associated bacterium to the latter species.

Keywords: *Agriotes* sp., *Rickettsiella* sp., Bacteria

Poster / Bacteria **Wednesday, 16:30 B-21**

Analysis of two toxic genes *xptA1* and *xptB1* in the entomopathogenic bacteria *Xenorhabdus nematophilus*

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Xenorhabdus nematophilus forms an obligate symbiosis with the nematode *Steinernema carpocapsae* that are high pathogenic to insects, thus currently used for biological control. This pathogenicity is mostly attributed to virulence factors produced by the bacteria. In this work we analyze the genectic variability of *xptB1* and the genetic expression of the genes *xptA1* and *xptB1* that belongs to a toxin cluster, in two distinct genetic groups of Azorean isolates. Genetic variability of the Azorean isolates was assessed by RAPDs and 16S rDNA sequence analysis. The variability of *xptB1* gene was obtained by comparing the *xptB1* nucleotide sequence of the Azorean isolates with 4 reference strains from America, Spain, France and United Kingdom. A Bayesian tree from mixed data showed that Azorean isolates form a genetic group, distinct from foreign isolates and that isolates from the east side of S. Miguel Island are separated from those of the west side. The variability in the *xptB1* gene indicates that Azorean isolates are different from the reference strains. Isolate Az157 has 39 difference aminoacid form United Kingdom strain. Gene expression determined by a quantitative *Real-time* RT-*PCR in* representatives of each Azorean clusters and *in the French strain showed that all the isolates expressed xptA1* and *xptB1* but at different levels. Strain. R1 had the highest level of expression of both genes whereas the lowest level was observed in the isolate Az157. It is being investigated how far these differences are related with bacterial virulence of each isolate.

Keywords: *Xenorhabdus nematophilus*, *xptA1*, *xptB1*, Genetic diversity, Genetic expression

Poster / Bacteria **Wednesday, 16:30 B-22**

Characterisation of an inhibitory compound (bacteriocin) from entomopathogenic *Bacillus thuringiensis* isolated from hazelnut beetle (*Balaninus nucum*)

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Bacteriocins are bacterial peptides that inhibit or kill microorganisms that are usually, but not always, closely related to the producer strain. It has been shown that the ability to synthesize one or more bacteriocins is a highly advantageous characteristic. In order to develop the potential use of the bacteriocine produced by the enthomopathogenic bacteria in the biological control, in this study, inhibitory compound (bacteriocin) produced by *Bacillus thuringiensis* isolated from hazelnut beetle, *Balaninus nucum* L. (Coleoptera: Curculionidae), has been determined and characterized. The bacteriocin production potential of the *Bacillus thuringiensis* was determined by testing on different identified bacterial species in agar spot method. The pH, temperature, organic solvent and enzyme resistance of partially purified inhibitory compound was characterized by well diffusion method. The results indicated that, *Bacillus thuringiensis* has inhibitory effects on 15 of 26 different indicator bacteria. Culture supernatants of *Bacillus thuringiensis* were sampled at various times during growth cycle and tested for bacteriocin activity. Production of inhibitory compound (bacteriocin) started during mid-logarithmic growth phase, reached a maximum at the mid-exponential growth phase and decreased during the late exponential growth phase of *Bacillus thuringiensis*. Application of Proteinase K observed the complete loose of the activity. No differences in the activity was determined with the application of 30°C, 50°C, for 30 min. However, 60

min at 50°C, 30 min and 60 min at 70°C observed the gradual decrease in the activity. 90 min at 70°C caused the complete loose of the activity.

Keywords: Bacteriocin, *Balaninus nucum*, Hazelnut Beetle, *Bacillus thuringiensis*

Poster / Bacteria Wednesday, 16:30 B-23

Genetic evidence of the protective role of the peritrophic matrix upon oral bacterial infection in *Drosophila melanogaster*

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The peritrophic matrix (PM) is an extracellular envelope composed of chitin and glycoproteins that lines the midgut of insects. The PM has been considered to be a physical and biochemical barrier, protecting the midgut epithelium from ingested pathogens and toxins. We previously reported that oral infection with a Gram-negative bacteria strongly induces the expression of genes which encode proteins with chitin-binding domains and which are annotated as putative components of the PM in *Drosophila*. This suggested that bacterial infection triggers the remodeling of this barrier, an as yet poorly characterized process that could be important in the defense against bacteria in the gut. Here, we have identified a loss-of-function mutation in an immune-inducible gene, named Insect Cuticle Protein C (ICP-C). ICP-C contains a signal peptide, a chitin-binding domain and putative glycosylation sites suggesting a role in PM formation. We show that ICP-C is expressed specifically in the midgut. By electron microscopy, we found that ICP-C mutant adults have a thinner PM than wild-type flies. Interestingly, the PM of ICP-C mutants is also more permeable than that of the wild-type as revealed by a FITC-dextran feeding assay. ICP-C mutants exhibit a higher expression level of antimicrobial peptides in the fat body upon oral infection with *Pseudomonas entomophila* and, crucially, these mutants die faster than wild type to such infections. Altogether, these results suggest an important role for the PM in the flies defense against ingested pathogenic bacteria.

Keywords: Peritrophic matrix

Poster / Bacteria Wednesday, 16:30 B-24

Degenerate PCR based search for genes encoding insecticidal proteins of Bacillus thuringiensis

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We have detected novel *cry* family genes encoding insecticidal proteins, referred to as Cry proteins, by degenerate PCR from known *Bt* strains, *Bt. galleriae* HD8, *Bt. tolworthi* H9 and *Bt. japonensis* H23, with insecticidal activities. Cry proteins contain eight highly conserved block regions, and degenerate primers were designed based on the targeting specific block regions. The DNA fragments amplified to the expected length using several designed primers were obtained. The analyses of nucleotide sequences and the deduced amino acid sequences revealed that the obtained nucleotide sequences were similar to *cry* genes. Some of them were confirmed as novel *cry* family genes by homology search in the DNA database of DDBJ with BLAST. One of the novel *cry* family genes detected from *Bt. japonensis* H23 was further analyzed to determine the open reading frame. As a result, deduced amino acid sequence of the nucleotide sequence contains block only 1-5 regions that are characteristic of the Cry proteins and homology of 81% to Cry1Bc. These results support that degenerate PCR is effective in searching for novel *cry* family genes of *Bt* strains.

Keywords: *Bacillus thuringiensis*, cry genes, degenerate PCR

Poster / Bacteria Wednesday, 16:30 B-25-STU

A study on the effect of sublethal doses of *Bacillus thuringiensis* on larvae of Colorado Potato Beetle, *Leptinotarsa decemlineata* (Say) (Col. Chrysomelidae)

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In this study the effects of sublethal doses of the varieties of *Bacillus thuringiensis* var. *tenebrionis* and *B.t.* var.*kurstaki* were investigated on second instar larvae of Colorado potato beetle feeding on the contaminated food until the development of adult insects. The results showed that in both cases, there was a meaningful difference between treated and control larvae during the first 8-day after bacterispray in terms of both growth trend and weight average. The lowest growth rate of larvae was observed in *B.t.* var. *kurstaki* treatment such that the larvae of this treatment had the lowest weight gain rate, that was 18.33 mg in average after 8 days, then *B.t.* var. *tenebrionis* treatment had the lowest growth rate with 30.50 mg in average weight after this time whereas the mean of weight of larvae in control treatment was 157.30 mg after 8 days. Also, there was a meaningful increase in the larval and pupal development periods in bactericide treatment group compared to the control treatment group. So that the highest larval and pupal mortality rate was related to the *B.t.* var. *kurstaki* treatment with 88 percent and the lowest mortality rate of larvae and pupae stages was observed in control treatment with 26 percent. Also the longest larval and pupal periods was observed in *B.t.* var. *kurstaki* with 30.18 days and the shortest periods of these stages was related to the control treatment with 19.44 days. The results from this study indicate that the total mortality caused by the application of *B. thuringieusis* based insecticides may be underestimated because high levels of mortality occurring primarily during later stages and the pupal stage in *B.t.* contaminated groups are often neglected

Keywords: Bacillus thuringiensis, Leptinotarsa decemlineata (Say) and Sublethal doses

Poster / Bacteria Wednesday, 16:30 B-26-STU

Investigation of interaction effects of *Bacillus thuringiensis* and Azadirachtin on third larval stage of *Plodia interpunctella*

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Indean meal moth (*P. interpunctella*) is an important stored-product insect specially for meal and storage wheat in the world. Along with aim decreasing uses of pesticides and fulfillment of biological control in the form of integrated pest management, the interactions between *Bacillus thuringiensis* var *kurstaki* (Biolep®) and azadirachtin (Neemazol®) were investigated on third larval stage of indean meal moth under laboratory conditions and on artificial meal. In this research work the interaction between bacterium and neem was assessed by using concentrations of *B.t.* and LC_{25} value of azadirachtin on third larval stage of *P. interpunctella*. The LC_{50} and LC_{25} values of resulted from the effect of *B.t.* and neem on third larval instar were 1871, 14297 ppm respectively. After determining the LC_{50} and LC_{25} of bacterium and neem, the combined effects of both agents were determined. For achieve this goal an experiment was designed in the form of completely randomized designed with treatment including concentrations of bacterium+LC_{25} of azadirachtin. Result of this experiment was remarkable. LC_{50} of *B.t.* was decreased from 1871 to 1249. Due to lower price of neem than *B.t.*, this decreasing of uses bacterium is economical. And because of faster effects of *B.t.* by this way, this method can help to decrease damage.

Keywords: *Plodia interpunctella*, *Bacillus thuringiensis*, Azadirachtin

Poster / Bacteria Wednesday, 16:30 B-27

***STU Bacillus thuringiensis* subsp. *kurstaki* (Btk) production and kinetics study in solid state fermentation with wheat bran and sugar cane bagasse**

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Bioinsecticides present great potential for the control of urban and agriculture plagues, as they are target-specific and innocuous to humans, differently from chemical defensives. This study evaluated the use of wheat bran and sugar cane bagasse to grow *Bacillus thuringiensis var. kurstaki* (Btk), which presents entomopathogenic activity against worms of the Lepidoptera order. Cultives were carried out using several rates, in dry basis, of wheat bran (FT) and sugar cane bagasse (BC) (100% BC, 100% FT, 30% BC + 70% FT, 40% BC + 60% FT, 50% BC + 50% FT, 60% BC + 40% FT and 70% BC + 30% FT) in different humidity ranges (40-45%, 50-55%, 60-65% and 70-75%). In addition, carbon source supplementation (glucose and sucrose), and addition of salts (K_2HPO_4, KH_2PO_4, $MgSO_4$, $MnSO_4$ $(NH_4)_2SO_4$ and $CaCO_3$) were studied. The higher growth - 10^9 CFU (colony-forming unit)/gms (gram of dry matter) – has occurred in the cultives without supplementation, in a culture medium composed by 60% wheat bran and 40% sugar cane bagasse, humidity of 65-70%. In such growing conditions, the log phase occurred around 30 hours, followed by maximum sporulation around 72 hours. It can be concluded that solid-state fermentation (SSF) has potential for Btk production, given the high sporulation rate, which can be associated to the toxins production, without the generation of residues to be treated, different from the submerged fermentation, which, despite the high sporulation rate, in the order of 10^9 and 10^{10} CFU/mL, generates effluents that require treatment.

Keywords: *Bacillus thuringiensis* subsp. *kurstaki*, solid state fermentation, bioinsecticides

Poster / Bacteria Wednesday, 16:30 B-28-STU

Study of the *Ostrinia nubilalis* Cry1Ab tolerance linkage to the *cadherin* locus

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Cry1Ab is the most widespread *Bacillus thuringiensis* Cry insecticidal toxin expressed in the transgenic maize, used to control *Ostrinia nubilalis*. As the susceptibility to these toxins in insect populations usually presents some variability, we have tested if a higher level of tolerance to Cry1Ab in some *O. nubilalis* individuals has genetic basis, and if it is linked to the *cadherin* locus, since this protein plays a relevant role in the mode of action of Cry toxins. One-hundred eighteen lines of single mating pairs derived from field collected individuals were established. Offspring of each line was treated with a Cry1Ab discriminating dose. A large variability in response was observed. The families with higher amount of survivors were used to establish F_2 generations, whose progeny were exposed again to Cry1Ab. A consistent reduced susceptibility to the toxin was found in five lines, where the segregation of the *cadherin* alleles was investigated with the Exon Primer Intron Crossing (EPIC) PCR approach. Significant differences among treated and non-treated individuals emerged in one line. The sequences of the putative toxin binding regions of the predominant alleles from the tolerant individuals were studied. No differences with sequences previously reported in literature were found, although the occurrence of mutations in other parts of the *cadherin* sequence cannot be discarded. The analysis of the results pointed out that the Cry1Ab tolerance trait in *O. nubilalis* has genetic basis, and that is linked to the *cadherin* locus (or to a locus near to *cadherin*) at least in some individuals.

Keywords: *Bacillus thuringiensis*, Cry1ab susceptibility, EPIC-PCR

Poster / Bacteria Wednesday, 16:30 B-29-STU

Testing alkaline phosphatase from *Heliothis virescens* as functional Cry toxin receptor

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Previous reports identified membrane-bound alkaline phosphatase from *H. virescens* (HvmALP) larval midgut as a putative Cry1Ac toxin receptor. The goal of this project was to test HvmALP as functional Cry1Ac receptor using RNA interference (RNAi). Occurring at the post-transcriptional stage, RNAi is a highly efficacious tool for testing gene function, and it involves the introduction of double-stranded RNA (dsRNA) molecules into a cell to degrade the mRNA complementary to the dsRNA sequence. To validate target specificity, we performed knockdown of stable HvmALP expression in *Drosophila melanogaster* S2 cell cultures using transfection with dsRNA targeting HvmALP. Effective knockdown of HvmALP expression in S2 cells validated our dsRNA to perform knockdown of HvmALP expression in *H. virescens* larvae. We used two approaches to deliver the dsRNA to cell in the larval midgut: egg microinjection, and feeding of dsRNA. Reduction of HvmALP expression levels in *H. virescens* larvae allowed us to test the role of this protein in Cry1Ac intoxication.

Keywords: Alkaline phosphatase, Cry1Ac, Heliothis virescens, RNAi

Poster / Bacteria Wednesday, 16:30 B-30-STU

Head-to-tail screening: An efficient approach to determine the activity–correlative regions of Cry proteins

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Crystal proteins, produced by *Bacillus thuringiensis* (Bt), is widely used in agriculture all over the world due to its specific insecticidal activity and harmless to human and animals. Insecticidal properties draw more and more attention and insecticidal specificity or activity become hot point in this field. Normally, site-directed mutagenesis and domain exchange are used to explore key amino acids or regions for specificity or activity. Some achievements were reached, such as some amino acids in loops are responsible for activity and domain Ⅲ is one of specificity determinants. However, the study of insecticidal specificity or activity is far away certainty; the race for key amino acids or regions is still on.

Here, we present a strategy of head-to-tail screening to identify the activity–correlative regions (ACR) of proteins by comparing the activities of the parent genes with those of the recombinants of a random and single-crossover recombination library (RSCRL) derived from 2 homologous parent genes with different activities. We developed a novel in vitro recombination method based on dideoxy random termination and re-extension with different templates to produce the RSCRL for screening. This method required several PCR procedures with DNA polymerases with different 3′→5′ exonuclease activity such as *KOD* DNA polymerase and *Taq* DNA polymerase. Using this method, we constructed an RSCRL of two Bt delta-endotoxin genes, *cry2Aa* and *cry2Ad*, which share 89.57% identity in DNA sequence and 86.10% identity in their encoded amino acid sequence. Subsequently, we performed bioassay and SWISS-MODEL analysis to identify 3 ACRs of Cry2Aa, which mediated toxicity to *Ostrinia furnacalis*, *Plutella xylostella*, and *Chilo suppressalis*, respectively. For *Ostrinia furnacalis*, the ACR is located in the region of 403S to 415S. For *Plutella xylostella*, the ACR is located in the region of 421Y to 439T. For *Chilo suppressalis*, the ACR is located in the region of 440R to 454S. The successful use of this novel method laid the foundation for further study, as well as broadened the channels for the study of relationship between protein structure and function. Furthermore, two Bt proteins with high insecticidal activity could be recombined by this method, to chimerize a new proteins with much higher insecticidal activity.

Keywords: Cry2 crystal proteins, gene recombination, key regions, structure, function

Poster / Bacteria Wednesday, 16:30 B-31-STU

The repeat region within Orf2 protein is a significant factor in Cry2Aa Toxin Crystallization

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Bacillus thuringiensis (Bt) is an important bacterium in biological control, which form a large crystalline inclusion containing insecticidal toxins in the mother cell of *Bacillus thuringiensis* during sporulation. It has been described that the formation of crystalline inclusion could utilize the cell space effectively. Cry2Aa is one insecticidal toxin that forming cubical crystalline inclusion during sporulation in *Bacillus thuringiensis*. *cry2Aa* is found in a three-gene operon which is made up of *orf1*,*orf2* and *cry2Aa* orderly. Crickmore (2001) reported that Orf2 was required for the crystallization of Cry2Aa toxin, it could be co-precipitated in the presence of Cry2Aa, suggesting a direct interaction between the two. Bioinformatics analysis showed that Orf2 is made up of two regions, one is non-repeat region（from 1M to 67N）, and the other is repeat region(from 68T to 185K). The repeat region contains 11 repeats, one of the repeats is made up of 15 amino acids (TYNQSQNVCPQDLVD). In the present work, we investigated the function of the repeat within Orf2 in the crystal formation. We constructed the mutants of orf2 with 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 repeats deleted in presence of the non-repeat region and the mutants with 0, 3, 4, 5, 7, 8, 9 and 10 repeats deleted in absence non-repeat region. All of the recombined plasmids were transformed into acrystalliferous strain HD-73$^-$ to examine the possibility of crystal formation and the expression level of Cry2Aa toxin. The results indicated that the repeat within Orf2 is important for cubical crystal

formation: while contains more than 6 repeats, the repeat region is capable of Cry2Aa crystallization in the absence of the non-repeat region; and also in the presence of the non-repeat region, the repeats (more than two) are necessary for the crystallization of Cry2Aa. The repeat region within Orf2 protein is a significant factor in Cry2Aa toxin crystallization.

Keywords: Repeat region within Orf2, Cry2Aa, crystallization

Poster / Bacteria Wednesday, 16:30 B-32-STU

Activation process of the coleopterancidal Cry8D on leaf-beetles

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Bacillus thuringiensis (Bt) is a rod shaped, gram positive, spore-forming bacterium. Bt produces parasporal crystal proteins during sporulation. Bt is widely used as pest control agents, the crystal proteins often show insecticidal activity to various harmful pests of Lepidoptera, Diptera and Coleoptera orders. Leaf-beetles are important Coleoptera pests because their leaf feeding causes serious damage to forest and crops. To Coleoptera larvae, Cry3, Cry7, Cry8, Cry43 toxins are known that they have specific toxicity. Among them, only Cry8D shows insecticidal activity against Japanese beetle not only larvae but also adults. We conducted bioassay using Cry8Da against various leaf-beetles and made it clear Cry8D have high insecticidal activity to some adult leaf beetle species such as alder leaf beetle, rice leaf beetle, and Japanese aspen leaf beetle. For example, alder leaf beetle, LC_{50} value was 559 μg/cm^2 (slope=1.99). We focused on processing patterns by midgut juice. Activation patterns of alder leaf beetle between Cry8Da and Cry8Ca were compared. (Cry8Ca have no toxicity against alder leaf beetles.) Cry8Da was activated and became 64kDa toxin, on the other hands, Cry8Ca was excessive degraded. This research demonstrated that some differences on the processing ways between Cry8Da and Cry8Ca affect insecticidal activity of alder leaf beetle.

Keywords: *Bacillus thuringiensis*, Cry8, leaf-beetles, coleoptera

Poster / Bacteria Wednesday, 16:30 B-33-STU

The first study on bacterial flora and biological control agent of fruit trees pest beetles *Sciaphobus squalidus* (Gyll.), *Tatianaerhynchites aequatus* (L.) and *Byctiscus betulae* L. in Republic of Moldova

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The weevils species *Sciaphobus squalidus* (Gyll.), *Tatianaerhynchites aequatus* (L.) and *Byctiscus betulae* L. (Coleoptera, *Curculionoidea*) are key pest of fruit crops in many parts of the world, in all Europe and Republic of Moldova inclusive. Present studies were conducted to identify their microbial gut flora which may provide novel approaches for insect control. We investigated the bacterial microflora of specimens collected from a range of host plants from spring 2008 through summer on the territory of the Republic of Moldova. Traditional culturing, morphological, physiological, biochemical and molecular techniques (16S rRNA PCR and sequencing) were used to characterize and identify microbial flora of these insects.
To date, all obtained isolates were identified as *Pantoea* sp. isolated from *B. betulae* and *S. squalidus*, *Enterobacter* sp. and *Staphylococcus devriesei* isolated from *T. aequatus*, *Bacillus cereus*, *Pseudomonas moraviensis* and *P. fluorescens* isolated from *S. squalidus*. This is the first complex study of bacterial microflora from fruit pest species *S. squalidus*, *T. aequatus* and *B. betulae* in Republic of Moldova. Our data can offer useful information for future investigations on bacterial agent development and implementation as insecticides in agriculture system of the Republic of Moldova. Further researches will be directed to investigate the potential role of these bacteria as biocontrol agents against crop pest beetles.
The study was made possible in part by Award no. MYSSP-1405 of The Moldovan Research and Development Association (MRDA) under funding from the US Civilian Research and Development Foundation (CRDF) in agreement with the Academy of Sciences of Moldova (ASM).

Keywords: *S. squalidus* (Gyll.), *T. aequatus* (L.), *B. betulae* L., bacterial flora, biological control

Poster / Bacteria Wednesday, 16:30 B-34

Characterization and pathogenic evaluation of *Bacillus thuringiensis* isolates from West Azerbaijan province-Iran

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In order to find native strains of *Bacillus thuringiensis*, toxic to some agricultural pests, a survey was conducted in West Azerbaijan province. Soil and other samples were taken and *B. thuringiensis* strains were isolated using acetate selection method with different concentrations. The morphology of crystals were studied using light microscopy. Bioassay tests were conducted on *Culex pipiens* and *Pieris brassica*. Based on the results, 48 *B. thuringiensis* strains were isolated from 736 samples. The best acetat concentration was determined as 0.25 M. Soil samples were the main source of B. thuringiensis (66%). Majority of strains (58%) had bipyramidal crystals. 22% of isolates showed toxicity against *Culex pipiens* and 44% against *Pieris brassica*. The results indicated Bt isolates showing insecticide activity can be used in integrated pest management to control agricultural and medical pests

Keywords: *B. thuringiensis*, isolate, *Culex pipiens*, *Pieris brassica*, insecticidal activity

Poster / Bacteria Wednesday, 16:30 B-35-STU

Isolation and diversity of *Bacillus thuringiensis* and insecticidal activity against red flour beetles (*Tribolium* castaneum)

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Different isolates of the soil bacterium *Bacillus thuringiensis* produce multiple crystal proteins toxic to a variety of insects; this research was performed to study the distribution and insecticidal activity of isolates of *Bacillus thuringiensis* from West Azerbaijan Iran. In our study, a total of 48 new isolates of *B. thuringiensis* that produce parasporal crystalline inclusions were isolated from 736 soil samples from different location by acetate selection method. Some of which show a potential to be used in biological control programs against coleopteran pests. Soil samples were the most abundant and diverse source of *B. thuringiensis* (%66). About 14.58% (7 isolate) and 18.75% (9 isolate) of the strains showed toxicity (>50% mortality) against larvae and adult *Tribolium confusum* respectively.

Keywords: *Bacillus thuringiensis*, isolate, *Tribolium castaneum*

Poster / Bacteria Wednesday, 16:30 B-36

Isolation and identification native *B. thuringiensis* in different habitat from west Azerbaijan and evaluate effects on Indian moth (*Plodia interpunctella*)

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The bacterium *B .thuringiensis*, Gram positive, aerobic, spore-forming is characterized by the production crystalline parasporal inclusion in stationary phase in sporulation growth stage with insecticide activity. This bacterium can be isolated and identified from different environment with navel toxin and can be evaluated toxicity against different host. In this report, 48 native strains isolated from 736 samples by acetate selection method in four concentrations (0.2, 0.25, 0.3 and 0.35 M.). Results showed more isolate obtained in 0.25 concentrations(56.26%), all isolates were characterized by crystal morphology, biochemical test and toxicity against 2nd instars Indian moth (*Plodia interpunctella)* larvae. 14 isolates had percentage mortality more than 75%. Isolates Wz-105, Wz-149, Wz-155, Wz-184 had percentage mortality (>90%) equal or more than *B. thuringiensis* kurstaki as positive control.

Keywords: *B. thuringiensis*, isolates, *Plodia interpunctella*

Poster / Bacteria Wednesday, 16:30 B-37-STU

Intramolecular proteolytic nicking and binding of *Bacillus thuringiensis* Cry8Da toxin in BBMVs of Japanese beetle

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Bacillus thuringiensis is a rod shaped, Gram positive, spore-forming bacterium. *B. thuringiensis* produces parasporal crystal proteins during sporulation. since the crystal proteins often show insecticidal activity to various harmful pests of Lepidoptera, Diptera and Coleoptera orders, *B. thuringiensis* is widely used in pest control agents. Larvae of the Japanese beetles live in soil and cause great damages to the roots of turf and other vegetations. On the other hand, adults damage the leaves, flowers and fruits but not roots. Cry8Da of *B. thuringiensis* have toxicity against both larvae and adult Japanese beetle. Cry8Da is processed into 64 kDa and 54 kDa with gut juice of Japanese beetle. The fragment of 54 kDa is derived from 64 kDa by further digestion at a loop between Alpha 3 and Alpha 4 helix, Domain I. Size exclusion chromatography couldn't separate 64, 54 and 8 kDa proteins. In addition the N-terminal sequence of 8 kDa protein is corresponding to the N-terminal sequence of 64 kDa protein. These suggest 54 and 8 kDa proteins are still forming part of the toxin complex. Further, co-precipitation binding assay revealed only the 54 kDa protein bound to both larvae and adult Japanese beetle BBMV. Intermolecular nicking at the loop between the Alpha 3 and Alpha 4 helix would be required to binding of Cry8Da and Japanese beetle BBMV. Ligand blot showed Cry8Da toxin bound to different proteins between larvae and adult Japanese beetle BBMV.

Keywords: *Bacillus thuringiensis*, Cry8D, Japanese beetle

COST 862

Poster / COST862 Wednesday, 16:30 BC-01

Occurrence of carabid beetles in Bt-maize fields

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Population dynamics of carabid beetles was studied at 6 localities in Slovakia. At each locality there were installed 3 pitfall traps in transgenic maize field expressing the Cry1Ab toxin from *Bacillus thuringiensis* (Bt-maize) and its isogenic hybrid. Three localities from eastern Slovakia (Záhor /N 48°38´ E 22°14´/, Palín /N 48°39´ E 21°59´/, Oborín /N 48°33´ E 21°53´/ and three localities from western Slovakia (Nitra – Krškany /N 48°17´ E 18°05´/, Nitra – Kynek /N 48°28´ E 18°02´/, and Trnovec nad Váhom /N 48°08´ 17°56´/) were selected for observation. Pitfall traps were installed on three dates and the insects were

collected after 2 weeks. The most usual carabid species were *Pseudoophonus rufipes* (De Geer, 1774) = *Harpalus rufipes*, *Calathus fuscipes* (Goeze, 1777), *Brachinus crepitans* (Linnaeus, 1758), *Anchomenus dorsalis* (Pontoppidan, 1763) = *Platynus dorsalis*. It was found that population of carabid beetles significantly depends on locality and time of collection. It was not found any influence of transgenic maize to population dynamics of Carabidae. This work was supported by Slovak grant agencies projects APVV COST-0043-06 and VEGA 1/0358/08.

Keywords: Carabid beetles, Bt-maize, Slovakia

Poster / COST862 **Wednesday, 16:30**
BC-02

E An alternative set of test to bioassay for bioinsecticides

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The development of an assay to determine insecticidal properties for either biological and conventional plant protection products plays an important role on the early screening of potential pathogens or derived toxins candidates. The standard methods for the evaluation it has been by bioassay, especially determination of LD50 or LC 50 requiring the use of relatively large numbers of insects and toxin tests. There are several problems connected with these bioassays: availability of insects and in the right life stage, mass producing the candidate species, preparation, reproducibly and costs relative to intensive manpower. These aspects are really important especially when bio-insectides should be tested against new target eg. invasive species in a new country. We present the results of the use of alternative systems in a screening study of *Bacillus thuringensis* (Bt) strain as potential pathogen for the Red Palm weevil, *Rhynchophorous ferrugineus.* We propose the use of different techniques such as growth inhibition, effect of pathogen on total number of hemocytes, ratio of selected hemocytes, espression of HSP 70 protein. In particular this protein is involved in the biochemical pathways response in stressed animals. Tests were carried out against the potential less sensitive stage to have a new predictive parameter for screening the reaction of the host to the pathogen. The preliminary results obtained with the proposed tests allows to enlarge the information of the potential pathogenicity of the entomopathogen bacteria and highlight the potential of this set of tests as screening methods to select new potential entomopathogens or bioinsecticides.

Keywords: Bioassay, Biological indicator, sublethal effect, *Bacillus thuringiensis*

Poster / COST862 **Wednesday, 16:30**
BC-03

Proteolytic processing of *Bacillus thuringiensis* Cry3Aa toxin is a key step in toxicity against Colorado Potato Beetle

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Previously we have shown that a Colorado potato beetle (CPB) ADAM metalloprotease recognizes and cleaves Cry3Aa toxin. We have now found that Cry3Aa proteolysis by the ADAM metalloprotease is a critical step in toxicity against CPB. Inhibition of Cry3Aa proteolysis by the metalloprotease inhibitor acetohydroxamic acid, the cleavage competitor pep-99 peptide or the lipid raft disrupting compound methyl-β-cyclodextrin correlated with a decrease in Cry3Aa toxicity against CPB larvae. Accordingly, an increase on Cry3Aa proteolysis by the calmodulin inhibitor trifluoperazine enhanced Cry3Aa toxicity in CPB dissociated midgut epithelium cells (MECs). In an attempt to localize the metalloprotease activity in the plasma membrane, we isolated CPB MECs detergent insoluble and soluble fractions by sucrose gradient ultracentrifugation. Cry3Aa toxin preferentially bound to the soluble fraction, although in the raft fraction a 36 KDa band was strongly immunodetected. The incubation of Cry3Aa with both fractions caused toxin proteolysis, but only the non-raft fraction yielded a 29 KDa fragment that was not produced in the presence of the metalloprotease inhibitor 1,10-phenanthroline, indicating that the ADAM metalloprotease localizes in this fraction. The proteolytic profile generated by the metalloprotease activity isolated in the non-raft fraction resulted slightly different compared to the one produced by whole MECs, suggesting that the interaction with the raft fraction might be needed for further cleavage by the ADAM metalloprotease. All these data evidence a compartmentalization of Cry3Aa toxin interactions in the plasma membrane, involving the association with lipid raft receptors and cleavage by a non-raft ADAM metalloprotease generating toxicity.

Keywords: *Bacillus thuringiensis*, Cry3Aa toxin, Colorado Potato Beetle, ADAM metalloprotease, proteolysis, toxicity, lipid rafts

Poster / COST862 **Wednesday, 16:30**
BC-04

Susceptibility to BT of wild lepidopteran species in Nature Reserve in Sicily

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A wide range of crop plants and trees have been transformed with genes derived from the soil bacterium *Bacillus thuringiensis* (*Bt*) to express insecticidal proteins (insect-resistant *Bt* plants). Whereas the adoption of *Bt* plants constitutes one of the most common uses of transgenic plants in agriculture, the environmental benefits and risks remain an issue. To preserve the natural reserve and their fauna UE proposed that in future the Member State specify a minimum separation distance of metres between fields of GM plants and nature reserves or to forbid cultivation of GM plant in particular area based on scientific data. Natural reserve in Sicily have many

endemism and rare species so next steps also in Italy should consider this before adaptation of GM crops. One first step is to obtain a listing of lepidopteran species that feed on these crops and their wild relatives, and to determine the host range of the larvae. Second to assess *Bt* toxin susceptibility for these lepidopterans. Only few species of Lepidoptera have been tested for susceptibility; and the literature suggests that generalizations about susceptibility among taxa are difficult due to the variability within families. However a similar approach could be interesting also for Bt as commercial product. We report the data on the susceptibility to Cry IAb and to Bt as spray for some not target lepidopteran species.

Keywords: Susceptibility, BT, lepidoptera

Poster / COST862 **Wednesday, 16:30**
BC-05

Activity of Cyt1Aa protein from *Bacillus thuringiensis* subsp. *israelensis* against the Mediterranean fruit fly, *Ceratitis capitata*

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Ceratitis capitata (Wiedemann), is one of the major threats to fruit culture worldwide. Current control methods rely mostly on synthetic insecticides. *Bacillus thuringiensis* (Bt) based-products should be a good alternative since they have been used for decades in controlling economically important pests. Our goal was to overcome the reported inefficiency of Bt preparations against *C. capitata*. Our first approach was to test *in vitro* solubilized crystal protoxins obtained from cultures of 35 native selected strains and 5 standard strains. A second level on δ-endotoxin processing was analysed; crystals from Bti were solubilized *in vitro* and thereafter incubated with gut extracts from 3 insect species (*C. capitata*, *Sesamia nonagrioides* and *Culex pipiens*). Protein electrophoresis revealed that protoxin activation was strongly dependent on the source of proteases. Dose-response assays showed that *in vitro* proteolytic processing of Bti protoxins increased lethal effects on *C. capitata* neonate larvae, being significantly higher when protoxins were predigested with *Culex pipiens* gut extracts. LC_{50} of *C. pipiens* digested Bti toxins was 31.26 µg/cm^2. Additionally, the use of a recombinant Bt strain allowed the identification a single δ-endotoxin, Cyt1Aa, responsible of the lethality found on *C. capitata* larvae. LC_{50} of this solubilized protoxin was: 4.93 µg/cm^2. We have shown that *in vitro* emulation of events of Bt mechanism of action may lead to increased activity against a recalcitrant pests such as *C. capitata*. The discovery of a single δ-endotoxin with larvicidal activity may be the basis for new engineered Bt strains effective on *C. capitata* control.

Keywords: *Bacillus thuringiensis*, *Ceratitis capitata*, bioassays, Bti, Cyt protein

Poster / COST862 **Wednesday, 16:30**
BC-06

Susceptibility of the 4th instar larvae of *Spodoptera exigua* (Hubner) (Lepidoptera: Noctuidae) to VIP3Aa toxin

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The beet armyworm (*Spodoptera exigua)* is a polyphagous pest which is effectively treated in the field at early larval stages with *Bacillus thuringiensis* based products. A vegetative insecticidal protein, Vip3Aa toxin, is expressed for some bacterial strains present in such products and it has been considered a relevant toxicity factor. Susceptibility of neonate *S. exigua* larvae to Vip3Aa has been reported, however other late larval stages are also exposed to intoxication in the field after spraying. In the present work, larvae from a laboratory population have been treated at the 4th instar stage with seven different concentrations of the toxin by surface contamination. The Vip3Aa toxin has been expressed in *E. coli* and the lysates were used in the assays. All tested larvae exhibited evidence of feeding after 24 h. At this time, a significant dose-dependant growth inhibition effect was observed compared to non-treated larvae. Moreover, it has been shown that pupation in control non-treated larvae took place in 5 days, but the Vip3Aa treatment produced a large increase of mortality and pupation failure. The results indicate a relevant fitness costs for *S. exigua* 4th instar larvae treated with the toxin and suggests that the toxin can be effective to control the pest in the field even for late instar larvae

Keywords: Growth inhibition, bioinseticides, Bacillus thuringiensis

Poster / COST862 **Wednesday, 16:30**
BC-07

Influence of Bt maize hybrids to predatory insects

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The occurrence of insects in yellow sticky traps installed in the fields of genetically modified (GM) maize expressing the insecticidal protein Cry1Ab from *Bacillus thuringiensis* (Bt maize) and its isomeric hybrid was observed at two localities in the southwest of Slovakia. In each variant there were used 12 yellow sticky traps on 6 different dates from June to September. It was found that the most abundant coccinellidae species in the maize fields were *Coccinella septempuncata* and *Propylea quatuordecimpunctata*. Populations of both species were the highest during the end of June and the beginning of July. Population dynamics of the other predators followed usually the population dynamics of

aphids. There was significant difference in the occurrence of predatory insects found at two localities. Bt maize hybrid did not influenced numbers of predatory insects in yellow sticky traps. But, the presence of Cry 1Ab toxin in maize tissues was lethal for the European corn borer larvae, which were not found in Bt maize plants.
This work was supported by Slovak grant agencies projects APVV COST-0043-06 and VEGA 1/0358/08.

Keywords: Bt maize, Coccinellidae, Chrysopidae, Nabidae

Poster / COST862 **Wednesday, 16:30**
BC-08

In vivo modulation of Hsp70 in *Rhynchophorus ferrugineus* hemocytes after *Bacillus thuringiensis* treatment

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Heat shock proteins (Hsps) are rapidly synthesized within stressed cells after exposure to an environmental stressor. A variety of environmental stresses, including heat, cold, trace-metal exposure, xenobiotics have been reported to modulate Hsps expression in various organisms. Hsps are grouped into several families based on their protein size. Most organisms have several genes encoding members of this Hsp family. In particularly Hsp70 can be induced quickly under stressful conditions, but return to a normal expression level under non-stressful conditions. Few studies have been done to detect the Hsp70 expression in phytophagous insects towards pathogens. Since a preliminary research disclosed that *Bacillus thuringiensis* (Bt) negatively interacts with *R. ferrugineus* circulating hemocytes so that their number was dramatically decreased. In the present research we examine the expression of Hsp70 in hemocytes from *R. ferrugineus* larvae feed with a commercial product based on Bt. Western blot analyses using monoclonal anti-HSP 70 antibody showed that the expression of Hsp 70 was modulated reaching the highest value, seven times highest to the control, after 3h from the treatment. The Hsp70 values had the same value of the control at 6 hours. Monitoring the Hsp70 for 48 hours we notice a further decrement. This result highlights a stress condition, caused by Bt, as showed also by the reduction of the larval weight. So Hsp70 may be a suitable tool to detect rapidly stress condition induced by potential entomopathogens.

Keywords: Hsp 70, Red Palm Weevil, environmental impact, biocontrol

Poster / COST862 **Wednesday, 16:30**
BC-09

Effects of Cry3Bb1-maize on the two-spotted spider mite

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Transgenic maize carrying the coleopteran-active *cry*3Bb1 gene from *Bacillus thuringiensis* proved to be effective in the control of the Western corn root worm (*Diabrotica v. virgifera*). Since the Cry toxin is expressed throughout the plant it could potentially has an effect on other herbivores. We assessed the impact of MON 88017 maize on the performance of the two-spotted spider mite, *Tetranychus urticae*, which is a serious pest of many crops in various ecosystems. Several parameters including developmental time, longevity, and fecundity were measured under constant laboratory conditions when spider mites were offered Bt or non-Bt maize leaves obtained from the field-trial experiment conducted in the vicinity of České Budějovice in 2009. The mean content of Cry3Bb1 toxin assessed using the ELISA test was 36.54 µg/g of fresh leaf tissue. The obtained results revealed that spider mites survived, developed and reproduced well on both Bt and non-Bt maize leaves. We can thus conclude that Cry3Bb1 has no toxic effect on *Tetranychus urticae*. This work was supported by NAZV grant No. QH91093 (2009-2011).

Keywords: *Bacillus thuringiensis*, toxin, transgenic plants, mites, Acari, *Tetranychus urticae*

Poster / COST862 **Wednesday, 16:30**
BC-10

Carboxy-terminal extension effects on crystal formation and insecticidal properties of Cry15Aa

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Cry15Aa protein, produced by *Bacillus thuringiensis* serovar *thompsoni* HD542, in a crystal together with a 40 kDa accompanying protein, is one of a small group of non-typical, less well-studied members of the Cry family of insecticidal proteins, and may provide an alternative for the more commonly used Cry proteins in insect pest management. In this study we examined the specific interaction between the C-terminal part of Cry15Aa and the 40 kDa protein for crystal formation in recombinant *B. thuringiensis* strain Bt51. Expression of the native cry15Aa operon in *B. thuringiensis* Bt51 resulted in formation of properly shaped crystals, while recombinant strains expressing full length Cry15Aa alone, or truncated versions of Cry15Aa with 40 kDa protein produced ovoidal inclusion bodies.
Both native and truncated versions of the Cry15Aa operon and of Cry15Aa alone were analyzed for crystal shape and insecticidal properties against the susceptible insect *Cydia pomonella - L* (Codling Moth). No significant differences in toxicity, before or after pre-solubilization of crystal spore preparations, were found.
Although the 40kDa protein significantly contributes to in vitro solubility and crystal formation of Cry15Aa, no direct evidence for involvement of the 40 kDa protein in toxicity of Cry15Aa was found.

Keywords: Cry15Aa, *Bacillus thuringiensis* serovar *thompsoni* HD542, *Cydia pomonella* (L)

Poster / COST862 **Wednesday, 16:30**
BC-11

Molecular characterization of putative receptors of the Bt toxin Cry3A in *Chrysomela tremulae*

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Chrysomela tremulae is a coleopteran pest of poplars which belongs to the same family as *Leptinotarsa decemlineata* and *Diabrotica virgifera*. It is susceptible to the Cry3A delta-endotoxin produced by some *Bacillus thuringiensis* (Bt) strains or expressed in transgenic poplars. We had previously performed high throughput sequencing on a *C. tremulae* cDNA library obtained after reverse transcription of larval gut RNAs. Among the sequenced cDNAs, we focused on those showing sequence homology with proteins described as receptors for Bt Cry toxins in various insects, i.e. cadherin and aminopeptidase N (APN). We now report the full-length sequencing of the cDNAs of the cadherin and three APNs present in *C. tremulae*. Moreover, mRNA expression levels of the different genes coding for these proteins were studied by qRT-PCR in different tissues and during development of *C. tremulae*. These data are discussed in terms of phylogeny and mode of action of Cry3A toxin in Coleoptera.

Keywords: Chrysomela, receptors, Cry3A

Poster / COST862 **Wednesday, 16:30**
BC-12

Effect of *Bacillus thuringiensis* on respiration rates of marine intertidal *Mytilaster intertidal, Mytilaster minimus* (Mollusca, Bivalvia)

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The development of an assay to determine insecticidal properties for either biological and conventional plant protection products plays an important role on the early screening of potential pathogens or derived toxins candidates. The standard methods for the evaluation it has been by bioassay, especially determination of LD50 or LC 50 requiring the use of relatively large numbers of insects and toxin tests. There are several problems connected with these bioassays: availability of insects and in the right life stage, mass producing the candidate species, preparation, reproducibly and costs relative to intensive manpower. These aspects are really important especially when bio-insectides should be tested against new target eg. invasive species in a new country. We present the results of the use of alternative systems in a screening study of *Bacillus thuringensis* (Bt) strain as potential pathogen for the Red Palm weevil, *Rhynchophorous ferrugineus.* We propose the use of different techniques such as growth inhibition, effect of pathogen on total number of hemocytes, ratio of selected hemocytes, espression of HSP 70 protein. In particular this protein is involved in the biochemical pathways response in stressed animals. Tests were carried out against the potential less sensitive stage to have a new predictive parameter for screening the reaction of the host to the pathogen. The preliminary results obtained with the proposed tests allows to enlarge the information of the potential pathogenicity of the entomopathogen bacteria and highlight the potential of this set of tests as screening methods to select new potential entomopathogens or bioinsecticides.

Keywords: Respiration rate, Bt, non target organisms, sublethal efects

Poster / COST862 **Wednesday, 16:30**
BC-13

Effectiveness and host range of endotoxin producing wild isolates *of Bacillus thuringiensis*

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Institute of Biology has been working on problems, which included development of microbiological methods for plant protection in Latvia for many years. The aim of the present study was to investigate the host range and effectiveness of endotoxins producing wild isolates of *Bacillus thuringiensis*. Pest insects used in tests: the codling moth *Cydia pomonella*, the fruit tree tortrix *Heydia nubiferana*, the apple ermine *Yponomeuta malinellus*, the gypsy moth *Lymantria dispar*, the cabbage white butterfly *Pieris brassicae*, aphids - *Aphis pomi* and *Schizaphis graminum*. Larvae of codling moth reared in laboratory on semi-synthetic media were used in experiments. Different amount of *B. thuringiensis* was added to nutrient media. Other insects were collected from nature, kept in the laboratory - in isolators under optimal conditions and reared on natural food, sprayed with different amount of Bt preparations. Effectiveness of isolates was expressed as the mortality percentage. Reasons of death were evaluated by direct examination of larval tissue smears under light microscope. Isolates were found those produce endotoxin Cry I and cause mortality of Lepidoptera. Identifications of cry1 geni were performed. This research has been financially supported by the grants from the Latvian Council of Sciences (09.1359).

Keywords: *Bacillus thuringiensis*, endotoxins, host range

Poster / COST862 **Wednesday, 16:30**
BC-14

Midgut microflora of sawfly, pine looper and gypsy moth with emphasis on *Bacillus* genus bacteria

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Entomopathogenic microorganisms play a major role in the population dynamics of many important forest pests, although little is known about the fundamental ecology of microorganisms in insect gut microflora.
The aim of studies was the detection and isolation of virulent strains of endotoxin producing *Bacillus thuringiensis* in significant forest pests and extend knowledge of (natural) gut microlfora of insects. As model organisms european pine sawfly (*Neodiprion sertifer*), pine looper (*Bupalus piniarius*) and gypsy moth (*Lymantira dispar*) were used.
Insects were collected from natural habitats in diferent regions of Latvia. After collecting living insects were placed in sterile isolators for observations. Contents of living insects intestine were homogenized and spread on artificial mediums. Morphologically different bacteria were isolated and classified by carrying on necessary chemical reactions: Gram staining, Oxidase test, Indole test. Bacteria were classified applying selective media, Crystal GP identification system, microscopy and imunofermentative reactions.
Results show that forest pests midgut microflora normally consisted of following bacterial geni: *Enterobacter*, *Stenotrophomonas*, *Corynebacterium*, *Agrobacterium*, *Sphingomonas* and *Bacillus*. Seven diferrent species of *Bacillus* genus were isolated from insects midgut.

Keywords: Forest pests, midgut microflora, *Bacillus* sp.

Poster / COST862 **Wednesday, 16:30**
BC-15

Resistance of *Plutella xylostella* to Bt and synthetic insecticides

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The diamondback moth (*Plutella xylostella*) is notorious for rapidly acquiring resistance to a large range of insecticides including the toxins produced by *Bacillus thuringiensis*. The nature of the resistance phenotype is often complex with evidence for the presence of multiple alleles, cross-resistance between seemingly unrelated insecticides and variations in the degree of dominance and sex-linkage in the resistance alleles. This presentation will consider three populations of *P. xylostella*: Karak – highly resistant to Bt toxins but no resistance to other insecticides such as the pyrethroid deltamethrin; Hosur – highly resistant to deltamethrin but not to Bt; SERD4 resistant to both Bt and deltamethrin. Data will be presented suggesting that multiple mechanisms of resistance to deltamethrin exist and that the genetic/physiological link between Bt and deltamethrin resistance in SERD4 is not present in the other two populations.

Poster / COST862 **Wednesday, 16:30**
BC-16

Expression of Bt toxins and their hybrids, and their toxicity towards *Plutella xylostella*

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E. coli expression vectors pGEM1Ah, pGEM1Ie and pGEM1Ac were constructed for the expression of *Cry1Ah*, *Cry1Ie* and *Cry1Ac* toxin genes. The genes were successfully expressed in *E. coli* JM109 under the control of the native promoter region of Cry1Ac. Cry1Ah is reported to have a broader spectrum of activity than Cry1Ac but has the problem of cross-resistance with Cry1Ac resistant insects. On the other hand, Cry1Ie has a narrower spectrum of toxicity but is able to overcome the problem of cross-resistance. Synergism studies were carried out between Cry1Ah/Cry1Ie and Cry1Ac/Cry1Ie against a Cry1Ac-resistant population of *Plutella xylostella* (KARAK). It was found that there was no synergism between these toxin pairs. To capture the broad spectrum advantage of Cry1Ah and the no cross-resistance property of Cry1Ie into a single toxin, hybrids CryAIA, CryIAI and CryIAA were constructed through domain swapping of Cry1Ah and Cry1Ie. CryAIA was successfully expressed in *E. coli* but CryIAI and CryIAA could not be expressed. However further characterization showed that CryAIA was not alkali soluble and did not leave a resistant core when treated with proteinases. It was also found to be not toxic to *Plutella xylostella*. Attempts to create N-teminally deleted forms of Cry1Ah and Cry1Ie also did not result in functional toxins.

Keywords: *Bacillus thuringiensis*, Cry1Ie, Cry1Ah, *Plutella xylostella*

VIRUSES

Poster / Viruses **Wednesday, 16:30 V-01**

Effect of host plant on the persistence of nucleopolyhedrovirus isolates of *Helicoverpa armigera* (HearNPV) under open weather conditions

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The rapid inactivation of viruses on foliage of plants is a limiting factor in their development as biological insecticides**.** To evaluate the effect of plant foliage on the virulence of seven HearNPV isolates and their relative persistence a set of experiments were conducted on tomato and cotton plants under open weather conditions. Results showed that, the virus lost its activity rapidly as the days of exposure to the weather condition on the host plants advanced. By the second day, more than 50 percent of the viral activity was lost on both plants and by the fourth day the

original activity remaining (OAR%) was reduced to about 20 percent on cotton and by another two days it fell to about 10 percent. Up to the fourth day, the NGM isolate recorded significantly higher OAR compared to the other isolates on cotton. On tomato also, the viruses lost their activity steadily as the days advanced. But it was interesting to see that from third day onwards the persistence of the virus isolates was significantly higher on tomato than on cotton. Comparison of the data of the two crops revealed that the persistence of the virus was higher on tomato than cotton. These results indicate that the virus may be used more efficiently for the management of *Helicoverpa armigera* on tomato than cotton. However, the problem of *H. armigera* is of greater concern on cotton since huge quantities of chemical pesticides are used on cotton. As a result of which, the NGM isolate could be considered as a relatively higher tolerate isolate on both the plants evaluated in the experiment.

Keywords: Nucleopolyhedrovirus, *Helicoverpa armigera*, persistence, tomato, cotton

Poster / Viruses **Wednesday, 16:30 V-02**

Natural occurrence of *Helicoverpa armigera* Nucleopolyhedrovirus and a fast screening of isolates derivated from tobacco farms of north of Iran

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The nucleopolyhedroviruses outbreaks are frequently declining the low density populations of tobacco bollworm *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) in tobacco farms throughout north of Iran. The HaNPVs isolates were derivated from dead and/or diseased larvae recorded and collected from the farms in Mazandaran and Golestan provinces during 2008-2009. The virus natural occurrence in the fields estimated. Among 227 samples, 29 have been selected for identification, propagation, extraction and purification of the nucleopolyhedroviruses. For a fast screening of the NPV isolates, 5 or 6-day-old larvae of *H. armigera* from a laboratory colony maintained on a semi-synthetic diet were treated by 1×10^3 OB/larva were added to a piece of diet for 24 hours feeding of each larva. The fast screening experiment has been done in a CRD containing 100 larvae for each isolate in three replicates at a temperature of 27±1 C and a relative humidity of 60±10%. That larva had consumed the whole piece of treated diet was transferred to untreated diet individually. Mortality percentage calculated by Schneider-Orelli formula after 6 days. The ANOVA showed a significant difference among the NPV isolates. The minimum value for NPV151 was 56.78% in group E of average comparison and the maximum values belonging to NPV10, NPV74, NPV108, NPV120, NPV167, NPV193 and NPV208 followed by 100, 98.99, 99.54, 98.78, 97.66, 97.56 and 95.67 %, respectively, in group A. It shows that virus isolates have high activity in lab as well as farms under the climatically conditions of north of Iran and further investigation is necessary.

Keywords: NPV, *Helicoverpa armigera*, Natural occurrence

Poster / Viruses **Wednesday, 16:30 V-03**

Possibilities of using baculovirus product Madex® for control of *Cydia pomonella* (L.), in Bulgaria

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The codling moth (CM), *Cydia pomonella* (L.), causes heavy damage in apples in Bulgaria. In spite of numerous treatments with chemical insecticides it presents a serious threat, due to development of resistance to insecticides. The intensive use of chemicals is also in a strong contradiction with principles of sustainable horticulture. Hence the urgent need appears for development of new control tools. The trials were carried out during the years 2006-2009 in South-Central and South-East Bulgaria. It was proved that with 4 treatments of Madex against the first and 6 treatments against the second generation, fruit damage and population density of CM may be maintained at a low level. Depending on the initial CM pressure in a particular orchard different control strategies have been suggested: (1) At a low population density (<1 larvae per tree, <1% of damaged fruits in previous year) 4-6 treatments with a half-dosage of Madex (50 ml/ha) against first and second generation; (2) at a moderate to high population density (1-3 larvae per tree, 1-5 % of damaged fruits) 4-6 treatments against first and second generation with a full dosage of Madex (100 ml/ha); (3) at a high population density (>3 larvae/per tree, >5% fruit damage) mating disruption + 4-6 treatments against the first and second generation with a full dosage of Madex (100 ml/ha). Madex may be a promising alternative to traditional programmes of CM control in contrast to chemical insecticides, it does not leave any residues on fruits.

Keywords: apple, codling moth, baculovirus Madex®

Poster / Viruses **Wednesday, 16:30 V-04**

Presence of nucleopolyhedrovirus in populations of the gypsy moth *Lymantria dispar* L. (Lepidoptera: Lymantriidae) in Latvia

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Forest pests have become a serious problem by causing damage in forestries during the last years in Latvia. Outbreaks of *L. dispar* were recognized in 2008 and 2009 near the city Liepaja. High defoliation (70 – 90%) of birches, alders and willows was observed. The aim of our studies was to search for

new isolates of nucleopolyhedrovirus (NPV) in *L. dispar* outbreak populations and to determine its biological and morphological characteristics. Dead, diseased, and living specimens were collected from natural habitats and checked for the presence of NPV by light microscopy. Living *L. dispar* were reared in the laboratory under optimal conditions (air temperature +20± 2°C, RH 75-85 %, photoperiod 16 h), provided with fresh natural food (leaves of food plants) or on semi-synthetic media. Rearing of larvae until pupation or death was followed by investigations of cadavers to confirm infections with NPV. All specimens were diagnosed by examination of fresh larval tissue smears, using phase contrast microscopy or fixed and stained with Giemsa. There were 500 asymptomatic larvae observed in laboratory, 22% of larvae survived and successfully pupated. Nucleopolyhedrosis, which was caused by LdMNPV, was detected in 46% of larvae.
Ultrathin sections, investigated by electron-microscopy, showed typical polyhedra with variable numbers of nucleocapsids per virion. Infections by LdMNPV were confirmed by using PCR and specific primers that correspond to the polyhedrin gene. The biological activity of LdMNPV was characterized by a bioassay using application with disc method.

Keywords: Nucleopolyhedrovirus, gypsy moth

Poster / Viruses **Wednesday, 16:30 V-05**

Spread of a nucleopolyhedrovirus within populations of balsam fir sawfly (*Neodiprion abietis*) larvae following its aerial application

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Field trials and assessments of the balsam fir sawfly nucleopolyhedrovirus (NeabNPV) against its natural host were conducted in July and August 2002 near Corner Brook, NL, Canada, in naturally regenerated, precommercially thinned stands dominated by balsam fir. Two experimental blocks, each with its own untreated control, were established. The purpose of the Island Pond block was to examine the spread of NeabNPV from a 313-ha aerial treatment block out into adjacent populations of balsam fir sawflies. The purpose of the Old Man's Pond block was to determine whether or not NeabNPV could disperse into populations of balsam fir sawflies within a 200-m zone between spray lines. NeabNPV was applied to treatment blocks by Cessna 188B AgTruck aircraft equipped with MicronAir AU4000 rotary atomizers at an application rate equivalent to 1×10^9 OBs/ha in 2.5L of 20% aqueous molasses. At Island Pond, NeabNPV infection increased with time following the spray ($F_{(3,48)}=81.53$, $p<0.01$), especially for individuals close to the treatment block and infection rate decreased with distance from treatment block ($F_{(5,48)}=12.93$, $p<0.01$). At Old Man's Pond, NeabNPV infection rose higher (80% versus 15%) and sawfly densities declined more (84% versus 60%) in the area between spray lines than in the control block.

Keywords: Nucleopolyhedrovirus, sawfly, Neodiprion

Poster / Viruses **Wednesday, 16:30 V-06**

Transmission of a nucleopolyhedrovirus within cohorts of balsam fir sawfly (*Neodiprion abietis*) larvae

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The spread of a nucleopolyhedrovirus (NeabNPV) within cohorts of balsam fir sawfly (*Neodiprion abietis*) larvae was studied by introducing NeabNPV-infected larvae into single cohort groups. In the laboratory, with populations equivalent to 1300 larvae/m^2 of balsam fir foliage, NeabNPV-induced mortality increased from 20% in control groups to over 80% with the introduction of one, five or 10 NeabNPV-infected larvae into treatment groups. In field studies, where populations were 220 larvae/m^2 of balsam fir foliage, NeabNPV-induced mortality increased from 23% in control groups to 51% with the introduction of one NeabNPV-infected, first-instar larva and to 84% with the introduction of 10 NeabNPV-infected, first-instar larvae. When one or 10 NeabNPV-infected, third instars were introduced into cohorts of third instars mortality was 60% and 63%, respectively. NeabNPV-induced mortality was higher when infected larvae were introduced into first- rather than third-instar cohorts. This was likely due to the greater susceptibility of early instars to NeabNPV and a longer period of time for the spread of the virus.

Keywords: Nucleopolyhedrovirus, sawfly, Neodiprion

Poster / Viruses **Wednesday, 16:30 V-07**

Physical characteristics of *Oryctes* virus that influence infectivity

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The *Oryctes* virus (OrV) has been used to control the coconut palm rhinoceros beetle (*Oryctes rhinoceros*) in the Pacific region for several decades. Although the *Oryctes* virus has had excellent success in reducing palm damage, a major drawback for use of this biocontrol agent has been the poor survival of the virus (outside of the beetle) under tropical field conditions. The *Oryctes* virus is a non-occluded virus that belongs to the provisionally formed Nudivirus family. Although loosely related to baculovirus, members of the Nudivirus family are not encased in a protective proteinaeous occlusion body, as is a common feature of some commonly used and well known nuclear polyhedrosis and granulosis virus. The unprotected state of the OrV means that it is readily degraded by UV, heat, detergents, and other environmental factors. The virus can be produced in cell culture and we have investigated the physical characteristics and features of the *Oryctes* virus that are important for maintaining infectivity. Results from these studies will assist in developing strategies and methods for improving field efficacy of the virus

against the coconut palm beetle. We will discuss our findings in relation to the continued use of this virus for control of the rhinoceros beetle.

Keywords: Oryctes virus, infectivity, biocontrol

Poster / Viruses Wednesday, 16:30 V-08-STU

Characteristic genomic features of the fast-killing *Epinotia aporema* Granulovirus

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Epinotia aporema (Lep. Tortricidae) is a major pest of legume crops in South America. A granulovirus isolated from *E. aporema* larvae (EpapGV) has demonstrated its properties as a fast-killing, type II granulovirus in our previous studies. In order to further characterize this virus, a draft sequence of its complete genome was obtained and its gene content analyzed preliminarily. The sequences were assembled in a 119.090 bp-long contig. The first analysis yielded a putative list of 141 ORFs that met the criterion of having a minimum of 50 codons and minimal overlap. They were compared with the GenBank sequences. The initial analysis focused on distinctive genomic characteristics compared to 12 granuloviruses sequenced so far. Blast searches revealed the presence of 31 baculovirus core genes as well as the GV-specific set. Additionally, some characteristic features include the presence of *chitinase*, *cathepsin*, *gp37*, *dUTPase* and *ribonucleotide reductase* subunits and the absence of *enhancin*. EpapGV genome sequence also revealed the presence of a new putative gene in the family *Baculoviridae*. This ORF encodes a 224 amino acid-long protein for which BlastP search resulted in multiple hits with tymidylate kinase-like proteins of several organisms. The nucleotide flanking sequences of this putative gene were analyzed. It appears to have an early promoter and a polyadenylation signal. This enzyme is involved in the dTTP biosynthesis and interestingly it is in the same biosythetic pathway to which ribonucleotide reductase and dUTPase belong, two genes present in some other baculoviruses.

Keywords: *Epinotia aporema*, granulovirus, genome

Poster / Viruses Wednesday, 16:30 V-09-STU

Development of a PCR based method for identification, discrimination and quantification of baculoviruses specific for cutworms, *Agrotis sp*.

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Cutworms of the species *Agrotis segetum* and *A. ipsilon* (Lepidoptera, Noctuidae) are serious pest insects in Africa, Europe and Asia, as they feed on many field crops and vegetables. In the past, four baculoviruses were isolated from *A. segetum* and *A. ipsilon* larvae and characterized on molecular level: Two nucleopolyhedroviruses (NPVs) were isolated from *A. segetum* larvae in Poland (AgseNPV-A) and United Kingdom (AgseNPV-B), one AgipNPV was found in *A. ipsilon* larvae and a granulovirus (AgseGV) was also isolated from *A. segetum*. Bioassays showed that both cutworm species are susceptible to all AgseNPV-A, -B, AgipNPV and AgseGV. To develop an environmentally safe biocontrol agent the narrow host range of baculoviruses is one of their advantages. For resistance management, however, the usage of a combination of different baculoviruses is regarded to be useful. Both requirements make AgseNPV-A, -B, AgseGV and AgipNPV excellent candidates as agents for the biological control of cutworms. In order to discriminate the different *Agrotis*-specific baculoviruses a reliable method for identification and quantification is essential. In this work, we present a multiplex polymerase chain reaction (PCR) and quantitative real time PCR (qRT-PCR) based method allowing the specific amplification of discriminating fragments of their polyhedrin (*polh*) and granulin (*gran*) genes. Thus, a rapid and robust method to detect the amounts of AgseNPV-A, -B, AgseGV and AgipNPV in mixed infections becomes possible and provides an important tool in quality control of production of baculoviruses specific for *Agrotis* species.

Keywords: Baculovirus, identification, *Agrotis segetum*, quantitative real time PCR

Poster / Viruses Wednesday, 16:30 V-10

Novel picorna-like virus from *Spodoptera exigua*

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Spodoptera exigua is a lepidopteran that currently represents a major pest of crops worldwide. While studying the transcriptome of *S. exigua* larvae by 454-based sequencing we identified several ESTs with homology to picorna-like viruses and with the highest similarity to infectious flacherie virus (*Iflaviridae*). Picorna-like viruses are a loosely-defined group of positive single strand RNA sharing a number of characteristics: a genome of around 10 kb with a protein attached to its 5' end, no overlapping ORFs, translated into a single polyprotein, and a conserved RNA-dependent RNA polymerase (RdRp).
Using sequence information derived from the obtained ESTs, we have completed the sequence of this virus. The novel *S. exigua* iflavirus (SeIV) has a genome of 10.3 kb and codes for a 3200 aa polyprotein. Structural and non-structural viral proteins and their cleavage sites were identified and compared with homologous sequences from other viruses from the *Iflaviridae* family. Presence of SeIV RNA has been detected in three geographically distant populations of *S. exigua*. The virus genomes from all three populations have been cloned, sequenced and compared. Unexpectedly, no polyA tail has been detected in their genomes. PolyA tail is usually needed by picorna-like viruses to replicate. However, we have obtained evidence

showing that replication is also possible due to a hairpin-like tertiary structure being used as initiation site for the negative strand replication. Additional studies have been also carried out in order to determine the viral abundance in different larval tissues as well as in response to larval exposure to different conditions.

Keywords: Iflavirus, picorna-like virus, *Spodoptera exigua*

Poster / Viruses **Wednesday, 16:30 V-11**

Analysis of Wuhan nodavirus genomic structure and characterization of it nonstructural protein B2

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Wuhan nodavirus (WhNV) was isolated from dead larvae of *Pieris rapae* infected by Granulosis virus (PrGV), in a cabbage field near Wuhan city, China. It was classified into the family Nodaviridae. The results of site-directed mutagenesis and western blot analysis indicated that the homologous Pr-E cells only synthesize B2-85 in the presence of WhNV RNA3. We conclude that the first AUG codon in WhNV RNA3 is the authentic translation initiation codon. Detection of fluorescent and RT-PCR showed that WhNV B2 protein could inhibit dsRNA and siRNA inducing RNAi in cultured Drosophila cell line S2. The amino-terminal region (aa 1-20) of B2 is essential for this RNAi inhibition activity. Furthermore, RNA binding and Dicer protection assays indicated that the proposal of a mechanism by which WhNV B2 inhibited RNAi at two distinct steps. First, by effectively coating long dsRNAs, B2 prevents their cleavage by Dicer and thus inhibits formation of siRNAs. Secondly, by virtue of its ability to bind tightly to siRNAs, B2 prevents incorporation of siRNAs into multiprotein RNA-inducing silencing complex (RISC) thereby inhibiting cleavage of target RNAs. We re-determined the terminal sequence of WhNV RNA1 and RNA2. 5'RACE and 3'RACE showed that WhNV RNA1 and RNA2 consisted of 3151 and 1572 nucleotides, respectively. The pWh1[G,0] and pWh2[G,0], with which the full length WhNV RNA1 and RNA2 containing the authentic termini could be transcribed, in the presence of T7 RNA polymerase, have been constructed. The results of RT-PCR indicated that the replicon and infectious cDNA clone of WhNV were constructed.

Keywords: Wuhan nodavirus, Subgenomic RNA3, B2 protein, RNAi inhibition, infectious cDNA clone

Poster / Viruses **Wednesday, 16:30 V-12**

Analysis of type 5 *Helicoverpa armigera* cypovirus genome

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HaCPV-5 has been able to be separated from the others through changing the rearing conditions of the *Helicoverpa armigera* larvae. Here S1 – 10 of HaCPV-5 genome were cloned and sequenced, thus the whole genome of HaCPV-5 has been completed. The positive strands of all the 10 segments showed conserved terminal sequences in both the 5' and 3' non-coding regions (NCR) (5'-AGUU...UUGC-3'). Computer analysis revealed that each HaCPV-5 genome segments contained a large open reading frame (ORF) that spanned >90% of the entire segment size. Analyzing the translation initiation codon of HaCPV-5 genome and available cypovirus sequences in GenBank, it was indicated that the conserved purine at the -3 position to AUG was probably the most important nucleotide for efficient translation initiation. Except for segments 5 and 10, all other segments of HaCPV-5 shared similarity to BmCPV-1, maybe this can give the reason for the similarity between the structure of their virions. The amino acid sequence comparison of S3 clearly showed that it shared similarity to RNA-dependent RNA polymerases coded by other reoviruses and the characteristic signature motifs for RdRp of Reovirus were also found in this segment, so HaCPV-5 S3 may code for RdRp. HaCPV-5 S5 contain a Poly (ADP-ribose) glycohydrolase (PARG) conserved domain and show similarity with some polyadenylate binding protein and ATPase which was involved in DNA repair. This similarity suggests the possibility of an exchange of genetic material between CPVs and other viral or cellular genomes at some point.

Keywords: Cypvirus, genome sequence, exchange of genetic material, RdRp

Poster / Viruses **Wednesday, 16:30 V-13**

Sequence analysis on the genome of the *Chroristoneura biennis* entomopoxvirus

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The two-year-cycle spruce budworm, *Choristoneura biennis*, harbours an entomopoxvirus (CBEV) previously characterized with respect to several of its genes and proteins. We determined that the CBEV viral genome is approximately 280 kb in size with 80% A+T residues. The "454" pyrosequencing technology was utilized to provide a more complete CBEV genome coverage. So far, 225 unique ORFs have been located, among which 49 were previously reported in all poxviruses and we reconfirmed that they are poxvirus core genes. By comparing the genomes of CBEV and that of *Amsacta moorei* entomopoxvirus (AMEV) (*Betaentomopoxvirus*), we determined their shared genes were collinear, especially in the central part of the genomes. A similar arrangement is found among chordopoxviruses. All of the 6 gene families reported for AMEV [17K ORF family, which contains the KilA-N; MTG (methionine-threonine-glycine) motif gene family; ALI-like (alanine-leucine-isoleucine) gene family; AMV176 gene family (unknown function); the tryptophan repeat gene family and the LRR (leucine-rich repeat) gene family] have homologues in CBEV. CBEV contains an ORF encoding for a ribonucleotide reductase small subunit that was reported in most chordopoxviruses. Interestingly, this gene is not present in the only two entomopoxviruses that have been fully sequenced so far, i.e. AMEV and

Melanoplus sanguinipes entomopoxvirus (MSEV). AMEV has 3 genes functionally related to NTPases/helicases. CBEV also encodes another NTPases/helicase-related protein s that was not found in the AMEV genome, but found in MSEV. While 454 sequencing did not provide a complete CBEV genome, attempts to fill the reminaing gaps and a more detailed analysis are underway.

Keywords: *Choristoneura biennis* entomopoxvirus, genome analysis, gene family

Poster / Viruses Wednesday, 16:30 V-14-STU

Sequence and genomic analysis of the *Mamestra brassicae* Nucleopolyhedrovirus-K1 isolated from Korea

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The nucleotide sequence of the *Mamestra brassicae* nucleoplyhedrovirus-K1 (MabrNPV-K1) DNA genome was analyzed and compared to those of other baculoviruses. Restriction endonuclease fragment analysis, using *Eco*RI, *Pst*I, and *Bam*HI, estimated that the total genome size of MabrNPV-K1 was about 150kb. To investigate more sequences of MabrNPV-K1, a degenerate polymerase chain reaction (PCR) primer set for the p10 gene was constructed by phylogenetic analysis results of the polyhedrin genes including previous reported MabrNPV-K1 polyhedrin gene. The sequence of partial p10 gene for MabrNPV-K1 was successfully amplified, and a total of 191 nucleotide sequences were determined. The nucleotide and amino acid sequences shared from 91 to 97% similarity with the p10 genes from *Helicoverpa armigera* MNPV (HearNPV), *M. configurata* NPV (McNPV) -B, McNPV-A 90/4 and McNPV-A 90/2. As the total genome sequence between HearMNPV and McNPV has a high similarity, various degenerate PCR primer sets were constructed from the comparison of two genome sequences and used for the amplification of MabrNPV-K1 DNA fragments. The gene parity plot analysis, percent identity of the gene homologues and a phylogenetic analysis suggested that MabrNPV-K1 is most related to HearNPV and McNPV, but with a distinct genomic organization. We present here the sequence and genetic organization of the MabrNPV-K1 genome.

Keywords: MabrNPV-K1, p10, polyhedrin, degenerate PCR

Poster / Viruses Wednesday, 16:30 V-15

AcMNPV LEF-2 is a Capsid Protein Required for Amplification but not Initiation of Viral DNA Replication

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The late expression factor 2 (lef-2) gene of baculovirus Autographa californica multiple nucleopolyhedrovirus (AcMNPV) has been identified as one of the factors essential for origin-dependent DNA replication in transient expression assays and has been shown to be involved in the late/very late gene expression. To study the function of lef-2 in the life cycle of AcMNPV, a lef-2 knockout mutant and repair bacmids were generated by homologous recombination in Escherichia coli. Growth curve analysis showed that lef-2 was essential for virus production. Interestingly, DNA replication assay indicated that lef-2 is not required for the initiation of viral DNA replication; rather, it is required for the amplification of DNA replication. lef-2 is also required for the expression of late and very late genes, as the expression of these genes were abolished in the lef-2 knockout bacmid. Temporal and spatial distribution of LEF-2 protein in infected cells was also analyzed and the data showed that LEF-2 protein was localized to the virogenic stroma in the nuclei of the infected cells. Analysis of purified virus particles revealed that LEF-2 is a new viral protein component of both budded and occlusion-derived virions, predominantly in the nucleocapsids of the virus particles. This observation suggests that LEF-2 may be required immediately after virus entry into host cells for efficient viral DNA replication.

Keywords: Baculovirus, AcMNPV, lef-2, knockout

Poster / Viruses Wednesday, 16:30 V-16

Expression and analysis of the baculovirus P10 protein in mammalian cells

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During the late phase of the baculovirus infection cycle, filament and tubule-like structures form in the host cell that are comprised of the virus P10 protein. Although the *p10* gene is highly conserved in the genus *alphabaculovirus*, to date no clear and defined function has been assigned to P10. Studies with AcMNPV have shown that P10 associates with the cellular microtubules and in the later stages, a P10 cage-like structure enwraps the nucleus. In order to determine if P10 structures require the presence of other virus proteins, and to examine further the role of microtubules in the formation of the P10 structures, we have expressed *p10* in mammalian HeLa cells under control of the CMV promoter. Using the *flash*BAC ULTRA™ system we have constructed a recombinant baculovirus encoding the *p10* and our initial findings indicate that p*10* expression can be facilitated in Hela cells and that initial P10-like structures form although these differ from the mature filament and tubule-like structures observed in virus-infected insect cells. This may suggest that other virus accessory proteins are required for the maturation of the P10 filaments into tubules or that the interaction with the mammalian microtubules is inefficient.

Keywords: AcMNPV, baculovirus, P10

Poster / Viruses **Wednesday, 16:30 V-17**

Stability analysis of many polyhedra variants of *Anticarsia gemmatalis* MNPV baculovirus

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In Brazil, baculovirus anticarsia is applied successfully for controlling the soybean caterpillar (*Anticarsia gemmatalis*), since early 80's. Virus production is mostly made by *in vivo* infection of caterpillars. Difficulties at *in vitro* large scale production are due to accumulation of mutants by serial passage in cell culture. Change from a parental many polyhedra (MP) to a few polyhedra (FP) per cell phenotype, presenting reduced occlusion and loss of virulence, is a major problem. However, a few infected cells still present stable viruses at high passages, MPs, which can be isolated by *plaque assay* technique. The attainment of stable MP variants is an innovative and valuable strategy for baculovirus *in vitro* production systems development. In the present work, two AgMNPV's MP viruses were evaluated for their genetic stability upon cell culture serial passages. MP variants monitoring show that, initially, the number of polyhedra for MP2 and MP5 was around 200 OB/nuclei (in the first passage), decreasing to minimum limits of 60-80 polyhedra per nuclei (7^{th} and 8^{th} passages). These data reveal that, despite the polyhedra/cell reduction, the resultant viral population of both MP variants in cell culture remained relatively steady, when compared to the original AgMNPV-2D previously gotten data. Serial passage of the AgMNPV-2D virus resulted in a great number of FP mutants, up from the 6^{th} passage, resulting on polyhedra production values of 30-40 OB/nuclei (7^{th} and 8^{th} passages). Characterization studies of these variants are in progress aiming their use for *in vitro* scale up production, such as in bioreactors.

Keywords: Baculovirus, Many Polyhedra, AgMNPV, Serial Passage

Poster / Viruses **Wednesday, 16:30 V-18**

Identification and preliminary characterization of a chitinase gene in the *Epinotia aporema* granulovirus genome

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The main function of baculoviral chitinase (V-CHIA) protein is to promote the final liquefaction of infected host larvae, facilitating the dispersion of the occlusion bodies in the environment. Among the twelve Granulovirus complete genome sequences available in the GenBank, only six appear to contain *v-chiA*. In this work, a *v-chiA* from *Epinotia aporema* Granulovirus (EpapGV) was identified and preliminarily characterized. The 1713 base-pair-long open reading frame (ORF) encodes a protein with a predicted molecular weight of 63 kDa. EpapGV CHIA sequence alignment resulted 61% identical to CpGV and AgSeGV V-CHIA, and Blastp search revealed high conservation among all baculovirus chitinases. Amino acid sequence analysis indicates that the C-terminal KDEL endoplasmic reticulum retention motif, which is conserved in most NPV chitinases, is absent in EpapGV V-CHIA, as also described for CpGV V-CHIA. The EpapGV *v-chiA* was cloned into a transfer vector which was co-transfected with a defective AcMNPV bacmid (bApGOZA) in order to generate a recombinant Ac-*chiA*EpapGV. Over-expression of chitinase was analyzed by SDS-PAGE and Western blot. Consistent with the absence of KDEL motif, it was detected in the supernatant at 48 h post-infection. Ac-*chiA*EpapGV polyhedra were purified, and the presence of chitinase was detected by Western blot. Enzyme activity assays in the infected cell supernatants were maximal between 27°C and 37°C. The enzyme remained active throughout the pH range 5-10. Finally, the effect of the over-expression of EpapGV chiA on peritrophic membranes and larval mortality response was evaluated.

Keywords: Chitinase, EpapGV, granulovirus

Poster / Viruses **Wednesday, 16:30 V-19**

Early gene *hhi1* of *Hz*NV-1 virus is a strong apoptosis inducer and crucial for latent viral re-activation

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Heliothis zea nudivirus 1 (*Hz*NV-1), previously known as *Hz*-1 virus, is an insect virus able to establish both productive and latent infections in several lepidopteran insect cells. It was previously regarded as an unclassified member of baculovirus family, and recently been placed to a new non-occluded genus, the *Nudivirus*. During productive viral infection, this virus generates for more than 100 transcripts in the infected cells. Here, we have cloned and characterized one of the *Hz*NV-1 early genes, *hhi1*, which maps to the *Hind*III-I fragment of the viral genome. In functional studies, we found that *hhil* can strongly induce apoptosis through the activation of caspase 3. Further experiments indicated that *hhi1* activates apoptosis through an inhibition of apoptosis 2 (Ac-*iap2*)-inhibitable cysteine protease pathway. Transient *hhi1* expression in latently-infected cells resulted in a significant increase in viral titer and viral DNA propagation, suggesting that *hhi1* plays a critical role in viral reactivation. Additional experiments showed that early genes, which possibly function in transcription or DNA replication, were activated in the latent cells upon *hhi1* transfection. Among these six genes, *orf90* and *orf121* expressions could be induced by *hhi1* alone without the need for other viral genes. Our discovery should be useful for future mechanistic study of the switches of latent/productive *Hz*NV-1 viral infections.

Keywords: HzNV-1, hhi1, apoptosis, latent infection

Poster / Viruses Wednesday, 16:30 V-20

Chikungunya virus nonstructural protein 2 is a potent inhibitor of JAK-STAT signaling

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Chikungunya virus (CHIKV) is an emerging human pathogen transmitted by mosquitoes. Like other alphaviruses, CHIKV replication causes general host shut off leading to severe cytopathicity in mammalian cells. Recent research, however, suggests that alphaviruses also fight the host antiviral response in a more specific manner. Here, we show that CHIKV actively suppresses the antiviral interferon (IFN) response by blocking STAT1 phosphorylation and/or nuclear translocation in VERO cells. Nonstructural protein 2 (nsP2) of related Sindbis and Semliki Forest viruses was previously shown by others to modulate the IFN response, however, a direct inhibition of the JAK-STAT pathway by nsP2 has never been demonstrated. Expression of individual CHIKV nsPs identifies nsP2 as a potent inhibitor of JAK-STAT signaling.

Keywords: Chikungunya virus, interferon, JAK-STAT, nsP2

Poster / Viruses Wednesday, 16:30 V-21-STU

Analysis of baculovirus gene function in insect cells

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Baculoviruses are arthropod-specific viruses, which contain a closed, circular, double-stranded DNA genome (80-180 kb). During baculovirus infection two structural forms are produced, budded virus (BV) and occlusion-derived virus (ODV). This study aims to elucidate the mechanisms involved in the production of ODV by deleting putative genes that encode ODV structural proteins. To study the function of the selected ODV structural proteins in the life cycle of AcMNPV, knockout mutant bacmids were generated using homologous recombination in *Escherichia coli*. Initial growth analysis showed that two of the deletion viruses (Δorf79 and Δodve28) showed reduced BV production and a third (Δodv-ec43) was not viable in cell culture. However, viability could be restored after a rescue assay with odv-ec43 fragment. The remaining deletion viruses (Δodv-e66, Δodv-e56. ΔCG30 and Δpif2) appeared to replicate normally in cell culture and comparative assays for BV production kinetics and cell viability were carried out with a non-deletion virus over a 96 hour time course. To determine the effects of these deletions on the production and occlusion of ODV, larva were injected with BV from one of the deletion mutant viruses and the occlusion bodies (OB) were extracted after death. These extractions have been prepared for transmission electron microscopy, to allow the ODV to be counted and viewed in OB.

Keywords: Baculovirus, occlusion-derived virus, occlusion bodies

Poster / Viruses Wednesday, 16:30 V-22-STU

Essential genes of BmNPV

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Nucleopolyhedrovirus (NPV) is a large family of DNA virus and that genome contains over 100 genes, which are expressed in a stage-dependent manner; immediate-early, delayed-early, late and very-late. Early genes are mainly involved in the regulation of the replication as transregulators and late genes are involved in viral structure. About half of them are predicted to be essential for viral propagation such as gene expression, DNA replication and virion assembly. However, some of their functions are still unknown. To understand viral replication mechanisms, it is important to reveal the functions of essential genes in viral replication process.
We constructed a series of gene-knockout BmNPVs (KOVs) for each of 136 genes using the BmNPV T3 bacmid system (Ono *et al.*, 2007) and lambda red recombination system (Datsenko and Wanner, 2000), followed by investigation of their growth properties in BmN cells. The knockout gene-specific effects on the infection spread and the polyhedrin gene expression were observed. Fifty two of the KOVs lacked the productivity of infectious budded virus (BV) but released varied amount of BV (viral DNA) into the culture medium. These observations suggested the possibility that the 50 genes were essential in different steps of the production of infectious BV. We here present some more data on the KOVs.

Keywords: BmNPV, essential genes, virus replication

Poster / Viruses Wednesday, 16:30 V-23-STU

***hr5* is a shut-off escaping element in the baculovirus-infected cells**

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Bombyx mori nucleopolyhedrovirus (BmNPV) is one of the major agents causing the lethal disease "nucleopolyhedrosis" in the silkworm. Recently, the transgenic silkworms possessing a transgene designed to transcribe dsRNA for suppressing essential viral genes were generated. However, the BmNPV-resistance enough in practical use has not been achieved. The moderate BmNPV-resistances observed for the transgenic silkworms were highly probably at least partly due to discontinued expression of dsRNA, which could be caused by BmNPV-induced shut-off of host gene expression (virus-induced shut-off). We here drew attention to an observation that the viral gene expression is not shut-off during viral replication, since the observation implies that virus has an element

escaping from virus-induced shut-off (Shut-off escaping element: SEE). We suspected *hr5* as the SEE since *hrs* scattering in viral genome were believed as important viral gene enhancing elements. We first constructed EGFP reporter plasmids which has a *hr5* and examined the SEE activity of *hr5* on the EGFP-expression in the BmNPV-infected cells, resulted in the observation that increased EGFP expression from the reporter plasmids with *hr5* in the BmNPV-infected cells. Subsequently, the recombinant BmN cells with the *hr5*-EGFP expression units was constructed and observed the enhanced expression of EGFP in the BmNPV infection while the transcripts from the endogenous GAPDH gene was markedly depressed. These observations suggested that *hr5* had the SEE activity both in the episomal plasmid DNA and in the cellular genome context. We then investigated the relationship between epigenetic modification of histones and the virus induced shut-off.

Keywords: Baculovirus, hr5

Poster / Viruses Wednesday, 16:30 V-24-STU

Molecular analysis of ORF AMV133 encoded by *Amsacta moorei* Entomopoxvirus (AMEV)

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Amsacta moorei entomopoxvirus (AmEPV) belongs to Poxviridae family, is an important insect virus. After analysis of complete genomic sequence of AmEPV, ORF AMV133 was suggested to be a putative triacylglyceride lipase gene. In this study, bioinformatic analysis shows that AMV133 have domains for a functional triacylglyceride lipase. Transcriptomic analysis of the gene was determined by RT-PCR and transcription start point (5'-UTR region of the gene) was determined by 5'-RACE. Transcription class was determined in infection of Ld652 cells in the presence of inhibitors of DNA or protein synthesis. Studies were performed on detection of the potential promoter sequences.
ORF AMV133 is transcribed at 6th hours post infection and has an early or early-late promoter. 5'RACE analysis showed that transcription initiated at position –77, relative to the translational start site of this gene. To determine the limits of the putative promoters, upstream sequences of various lengths were cloned in front of a firefly luciferase reporter gene. The resulting plasmid constructs were tested in a dual assay. The promoter activity was lost when the length of the sequence upstream of the translational start site was reduced from 82 to 21 nucleotides. Our results show that it is an active gene and plays function in virus replication. Therefore, our future studies directed to determine the function of this gene in virus replication.

Keywords: *Amsacta moorei* entomopoxvirus, lipase, transcriptomic, promoter analysis

Poster / Viruses Wednesday, 16:30 V-25-STU

The AcMNPV *ptp* gene induces hypermobile behavior in *Spodoptera exigua* larvae

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'Wipfelkrankheit' is a phenomenon observed in lepidopteran insect larvae upon infection with a baculovirus. Infected host larvae show a behavioral change, characterized by hypermobility and climbing to the top of the plant. This host behavior is thought to be beneficial for the virus as it enhances the spread of progeny virions. *Spodoptera exigua* larvae became hypermobile three days after infection with *Autographa californica* (Ac) MNPV. In contrast, a mutant AcMNPV in which the *ptp* gene was deleted (Δptp), did not induce this hypermobility. To further study the role of the *ptp* gene in larval hypermobility, three additional recombinant baculoviruses were constructed: (i) a repair virus, in which the *ptp* gene was re-inserted in the AcMNPV genome, to use as a control, (ii) a virus carrying a *ptp* gene that encodes a catalytically inactive enzyme to determine whether the phosphatase activity of the PTP protein is required for the induction of hypermobility, and (iii) a virus that carries *Spodoptera exigua* MNPV *ptp2* instead of the AcMNPV *ptp* gene to study whether PTP2 is a functional homologue of PTP and can also induce hypermobility. Bioassays were performed in early third-instar *S. exigua* larvae to determine the LD_{50} and LT_{50} of these viruses. Currently the three newly-engineered viruses are being tested in movement assays of infected larvae, in parallel with the wt and Δptp AcMNPV strains. The results will contribute to our understanding of the mechanism behind *ptp*-induced host hypermobility brought about by baculovirus infection.

Keywords: Baculovirus, behavior, ptp

Poster / Viruses Wednesday, 16:30 V-26

A new baculovirus vector for expression of foreign genes in the *Lymantria xylina* larvae and cell lines

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A new baculovirus expression vector derived from *Lymantria xylina* nucleopolyhedrovirus (LyxyMNPV) required for the expression of foreign genes in the *L. xylina* cell lines and caterpillar hosts was established. The gene encoding the coral red fluorescent protein (DsRed) under the polyhedrin (*polh*) promoter was introduced into LyxyMNPV by homologous recombination. The resulting recombinant LyxyMNPV isolate was purified by plaque assays and named vLxDsRed. Via the time course of DsRed expression *in vitro*, LyxyMNPV *polh* promoter proved to be a very late promoter that can efficiently drive foreign gene expression. On the other hand, vLxDsRed was capable of causing a seriously systemic infection *in vivo*, and expressed a huge amount of DsRed protein comparable to that of the

polyhedrin protein expressed from the wt-LyxyMNPV-infected *L. xylina* larvae. The identity of the DsRed protein was confirmed by both copper binding study and Western blot analysis. These results demonstrated that this new baculovirus expression system has a potential to use not only for foreign gene expression but also for the control of *L. xylina* populations.

Keywords: Recombinant baculovirus, *Lymantria xylina*, red fluorescent protein, insect cell line, expression vector, DsRed

Poster / Viruses Wednesday, 16:30 V-27-STU

Construction of recombinant baculoviruses without using cell cultures

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Homologous recombination in cultured insect cells is a critical step in the classical construction of recombinant baculoviruses, which are important tools for elucidating viral gene function and infection processes. However, except for a few model systems including *Autographa californica* nucleopolyhedrovirus (NPV) (AcMNPV) and *Bombyx mori* NPV, most of the ~600 known baculoviruses lack permissive cell lines and, consequently, their molecular pathology is poorly understood. In this study, we examined how to construct recombinant NPVs *in vivo* without using cell culture. First, appropriate conditions for injecting wild-type virus DNA into insect larvae were determined. Second, the production of recombinant AcMNPV was compared *in vivo* and *in vitro*. Transfer vectors including a marker gene, lacZ, were co-injected or co-transfected with wild-type AcMNPV DNA into *Spodoptera exigua* larvae or Sf-9 cells, respectively. Homologous recombination was assessed by both beta-galactosidase assay and PCR using lacZ-specific primers. Successful marker gene insertion was detected in the *in vivo* system, but beta-galactosidase activity in either cell lysates or insect homogenates did not always correlate with lacZ gene insertion as detected by PCR. Finally, end-point dilution and *in vivo* inoculation will be conducted to isolate recombinant genotype from wild type. Appropriate method for *in vivo* construction of recombinant baculoviruses would be discussed.

Keywords: Recombinant, baculovirus, *in vivo*

Poster / Viruses Wednesday, 16:30 V-28-STU

Enhanced production of CSFV E2 protein by fusion expression with partial polyhedrin of nucleopolyhedrovirus

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The Classical Swine Fever Virus (CSFV) is a member of the *Pestivirus* genus of the *Flaviviridae*. The polyprotein composed of eight nonstructural and four structural proteins (nucleocapsid protein C and three envelope glycoprotein E0, E1 and E2). E2, the most immunogenic of the CSFV glycoproteins, induces a protective immune response in swine. The objective of this study was to enhance production of E2 protein by fusion with partial polyhedrin of nucleopolyhedrovirus in insect cells. We generated various E2 form by fusion with different combinations of the partial polyhedrin and deletion of the C-terminal transmembrane region (TMR). Expression of the E2 protein was identified by SDS-PAGE and Western blot analysis using anti-His Tag and anti-CSFV E2 monoclonal antibodies. The fusion expression of an E2 protein with the partial polyhedrin markedly increased expression levels. Also, expression of E2 protein lacking TMR region was higher than that of intact E2 protein. As a result, the fusion expression of E2 protein lacking the C-terminal TMR with partial polyhedrin was significantly increased in insect cells. These suggest that the fusion of target foreign protein with partial polyhedrin could enhance significantly the production of target protein.

Keywords: Polyhedrin, BEVS

Poster / Viruses Wednesday, 16:30 V-29

Baculovirus-expressed haemagglutinin (HA) of an influenza H5N1 virus as a diagnostic reagent

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Baculoviruses are often used to generate foreign proteins including as protein reagents in diagnostics of viral infections. Recently influenza virus H5N1 has become of concern in both birds and humans and different vaccines are being developed against H5N1 infections. The HA protein is a major influenza virus membrane protein (the others are neuraminidase and M2 ion pore protein). In influenza virus infected cells, the 75 kDa HA0 is proteolytically (subtilisin) cleaved at a multibasic site into 55 kDa HA1 and 25 kDa HA2 fragments. We developed a recombinant fowl adenovirus vaccine (FAdV-HA) expressing the H5 haemagglutinin gene as evidenced by Western immunoblotting, RBC adsorption to FAdV-HA infected cells and haemagglutination inhibition (HI). To measure the antibody response to HA following immunization with FAdV-HA we needed an HA reagent for use in immunological assays. Since H5N1 influenza virus requires high containment, it was not a suitable reagent for this purpose. To this end we cloned the H5N1 influenza virus (his tagged) HA gene into an AcMNPV bacmid-derived virus (AcMNPV-HA). Expression of HA by AcMNPV-HA was monitored by Western immunoblotting using a commercial anti HA monoclonal antibody which revealed a positive band migrating at about 37 kDa but which was absent in control lanes of uninfected and wt AcMNPV infected cells. The baculovirus-expressed HA was used for Western immunoblotting to test sera from birds vaccinated with the FAdV-HA. Preliminary results by Western immunoblotting indicated that sera from FAdV-HA vaccinated birds resulted in positive

bands against cell extracts from AcMNPV-HA-infected cells.

Keywords: Baculovirus expression vectors influenza virus haemagglutinin

Poster / Viruses Wednesday, 16:30 V-30

Chikungunya virus glycoprotein expression by recombinant baculoviruses in insect cells

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Chikungunya virus (CHIKV) is a mosquito-borne *alphavirus* causing epidemics in the Indian Ocean region. Due to the expanding distribution of its mosquito vector *Aedes albopictus* CHIKV is rapidly spreading to other parts of the world including Europe. Humans infected with CHIKV may suffer from high fever, severe joint pain, skin peeling and long lasting arthritis. Control or mitigation strategies of the disease are required. CHIKV has gained renewed interest due to the large-scale epidemic on Reunion Island in 2006, where almost 40% of the total population became infected. In 2007, CHIKV reached the European continent where it was spread by local *Aedes albopictus* mosquitoes in Italy. Currently, no CHIKV vaccine or antiviral treatments are available. For other alphaviruses neutralizing antibodies are generally directed against the two envelope-glycoproteins E1 and E2. In this research, recombinant baculoviruses were constructed encoding genes for CHIKV-E1 and -E2 glycoproteins with or without C-terminal transmembrane domains. All proteins were expressed at high levels in Sf21 insect cells and could readily be detected by Western blot using a polyclonal CHIKV antiserum. The potential use of baculovirus-expressed CHIKV-E1 and -E2 to induce neutralizing antibodies and to develop a vaccine will be discussed.

Keywords: Chikungunya virus, vaccine, glycoproteins, baculovirus

Poster / Viruses Wednesday, 16:30 V-31-STU

Cloning and expression of the glycoproteins gB, gC, and gD of Aujeszky's Disease Virus NYJ strain in *Bombyx mori* cells and larva

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In order to characterize the Aujeszky's disease virus (ADV) NYJ strain isolated from the serum of an infected pig in Korea, nucleotide sequence of three major glycoproteins (gB, gC, and gD) were analyzed and the expression of glycoprotein gD was investigated using baculovirus. As a result, the glycoproteins gB, gC, and gD of NYJ consisted of 2751 bp, 1443 bp, and 1203 bp, respectively, and these had identity ranging from 94.2 to 99.8% with other strains. To better understand the genetic relationships between other strains, phylogenetic analyses were performed. The NYJ strain was formed a distinct branch with high bootstrap support. The expression of glycoprotein gD in Bm5 cells and silkworm, *Bombyx mori* was performed using novel transfer vector, pBmKSK4 which has the polyhedrin promoter of BmNPV and 6x His tag. Glycoprotein gD of approximately 45 kDa was detected specifically in both Bm5 cells and silkworm larvae by His tag and porcine anti-ADV antibodies. The results of this study have implications for both the taxonomy of ADV and vaccine development.

Keywords: BEVS, ADV, *Bombyx mori* larvae, Bm5 cell

Poster / Viruses Wednesday, 16:30 V-32-STU

Deacetylation of GD3A using baculovirus-a novel therapeutic approach for invasive glioma

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Tumours of the central nervous system rank second only to leukaemias as the biggest childhood killer, aside being the leading cause of death for all childhood solid cancers. Neurosurgery, chemotherapy and radiotherapy have increased survival times of paediatric brain tumours; however, they surface after therapy. Thus the need for new therapies targeting the infiltrating anaplastic cells is paramount.

A ganglioside-GD3 is highly expressed during embryonic development and involved in cell migration, adhesion and induction of apoptosis in excessively proliferating cells. GD3 expression markedly decreases in latter stages of development; however, its expression is upregulated in neoplastic cells, regulating tumour growth and invasion. Accumulation of GD3 in non-neoplastic cells induces mitochondrially-mediated apoptosis, whilst the acetylation of GD3 to form GD3A in tumour cells prevents apoptosis. GD3A enhances the invasive potential of the tumour cells as well as promoting their survival. The aim of this study was to convert GD3A to GD3 via exogenous esterase treatment and using baculovirus to deliver esterase gene.

Baculovirus is now considered a novel vector for gene therapy since it does not replicate in mammalian cells but efficiently transduce a wide range of mammalian cells. It evokes less of an immune response compared to adenovirus.

In vitro deacetylation of GD3A via exogenous esterase treatment of gliomas resulted in a decreased expression of GD3A (47%) and an increase in the expression of GD3 (18.43%). Recombinant baculoviruses that express a haemaglutinin esterase gene in human glioma models have been generated and characterised for further study.

Keywords: Gliomas, GD3/GD3A, baculovirus, acetyl esterase

Poster / Viruses Wednesday, 16:30 V-33

A deletion virus for improved recombinant protein expression

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The baculovirus expression vector system (BEVS) uses virus-infected insect cells to produce recombinant proteins to high levels, and these are usually processed in a similar way to the native protein. Interestingly, since the development of the BEVS, the virus most often used (*Autographa californica* multi-nucleopolyhedovirus; AcMNPV) has been little altered genetically, from its wild-type parental virus. In this study, we modified the AcMNPV genome in an attempt to improve recombinant protein yield, by deleting genes that are non-essential in cell culture. We deleted the *v-cath, chiA, p26, p10* and *p74* genes from a virus genome, replacing them with an antibiotic selection cassette, allowing us to isolate recombinants. We screened and identified recombinant viruses by restriction enzyme analysis, PCR and Western blot. Cell viability analysis showed that the deletions did not improve the viability of infected cells, compared to non-deletion viruses. However, expression studies showed that recombinant protein levels for the deletion viruses were significantly higher than the expression levels of non-deletion viruses. These results confirm that there is still great potential for improving the BEVS, further increasing recombinant protein expression yields and stability in insect cells.

Keywords: Recombinant protein expression

Poster / Viruses Wednesday, 16:30 V-34-STU

Identification of proteins associated with *Helicoverpa armigera* Nucleopolyhedrovirus budded virions

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The budded virion (BV) of baculovirus is responsible for secondary infection, spreading the virus between cells and tissues in permissive hosts. In this study, we analysed the protein composition of the BV of *Helicoverpa armigera* Nucleopolyhedrovirus (HearNPV). Proteins from purified HearNPV BV were urea-denatured or separated by SDS-PAGE gel, subjected with trypsin digestion and then introduced to quadrupole-linear trap mass spectrometer (Qtrap MS/MS) and quadrupole time-of-flight mass spectrometry mass spectrometer (Q-TOF MS/MS) respectively. Totally, 36 proteins were identified to be associated with HearNPV BV, 24 of which were both identified by two tandem MS/MS methods. Summary with references, 39 proteins with HearNPV BV were identified, including 25 proteins were newly identified as structural proteins of HearNPV BV. Three and twelve proteins have been previously determined as envelope and nucleocapsid proteins respectively. A comprehensive differential localization of protein within HearNPV BV will be revealed by iTRAQ labelling and quantitative MS analysis, western blot and immunoelectron microscopy. Our study would increase our understanding of compositions of BV and ODV and promote further proteomic studies on all baculoviruses, thus facilitate understanding of BV/ODV assembly and maturation and mechanism of baculovirus infection.

Keywords: *Helicoverpa armigera* Nucleopolyhedrovirus, Budded Virions, Proteomics

Poster / Viruses Wednesday, 16:30 V-35-STU

Functional analysis of N-linked glycosylation of *Helicoverpa armigera* Single Nucleocapsid Nucleopolyhedrovirus envelope fusion protein F

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The F protein is the major envelope fusion glycoprotein of *Helicoverpa armigera* (Hear) Nucleopolyhedrovirus. Glycosylation is important for glycoprotein functioning, but in the case of HaF, only one N-glycosylation site located on the F_2 subunit (Asn104) was characterized, and the importance for F functioning needs further in-depth investigation. Five potential glycosylation sites were predicted on the HaF_1 subunit (Asn_{293}, Asn_{361}, Asn_{526}, Asn_{571} and Asn_{595}). A series of recombinant HearNPVs carrying N-linked glycosylation sites mutated HaF were generated. All five single mutants produced infectious BVs, but only the three conserved sites (Asn_{293}, Asn_{526} and Asn_{571}) showed a downshift in size of F suggesting the presence of a glycan group. The mutants with multiple mutations in F were viable, but produced less BVs. The F_1 Asn_{293} exhibited enhanced fusogenicity than WT HaF, whereas the double, triple and quadruple mutants were less fusogenic or not fusogenic at all. Moreover, the lack of glycosylation seems to impair the transport of HaF from the ER/Golgi complex to cell surface. Although N-glycosylation of F protein is not essential for the HearNPV BV infection process, it does plays a role in F protein functioning. The role of F protein glycosylation in baculovirus pathogenesis will be further discussed.

Keywords: Baculovirus, F protein, N-glycosylation

FUNGI

Poster / Fungi Wednesday, 16:30 F-01

Effects of certain pre-incubation and incubation conditions on the Zygospore germination of *Conidiobolus osmodes* Drechsler (Arcylistaceae: Entomophthorales)

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Amongst entomopathogenic fungi, entomophthoralean species are commonly effective on natural insect populations as a suppressing factor. One of limiting factors on their use in microbial control practices is insufficient knowledge of the conditions required for germination of their resting spores, the spores resistant to unfavorable conditions. In this study, effects of certain incubation and pre-incubation conditions on germination of *Conidiobolus osmodes* Drechsler zygospores were investigated in controlled conditions. All experiments were conducted according to complete randomized experimental design with three replications. Distilled water agar was used for germinating the spores. According to the results; (1) ambient temperature was an important factor on germination and amongst the tested temperatures, 16 ^{o}C was the most suitable with the germination rate of 3.14 % per day, (2) amount of agar in the medium used for germination affected germination and the best medium including 1.5 % agar supported 99.00 % germination within 46 days, (3) zygospore germination was reduced to 11.33 % under continues light conditions, (4) vernalisation was not required for germination and when it was prolonged (5 weeks) the spores were deactivated, (5) germination rate of older zygospores and those dried prior to incubation was higher but time required for their germination was longer, (6) even low amount of citric acid, oxalic acid, ethyl alcohol and Tween 80 had negative effect on germination. Further studies are required to understand the extend of validity of these results amongst species in Entomophthorales, especially those in the genus *Conidiobolus*.

Keywords: Entomopathogenic fungi, microbial control, zygospor germination, environmental conditions

Poster / Fungi Wednesday, 16:30 F-02

Extraction and characterization of extracellular protease from the entomopathogenic fungus, *Beauveria bassiana* in the presence of *Eurygaster integriceps* (Hemiptera: Scutelleridae) cuticle

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The extracellular protease from the entomopathogenic fungus, *Beauveria bassiana* in the presence of *Eurygaster integriceps* (Hemiptera: Scutelleridae) cuticle was isolated, purified and characterized. Isolate B1 of *B. bassiana* that shows high virulence against *E. integriceps* was examined for the production of the cuticle-degrading proteases. Results showed that both subtilisin-like (Pr1) and trypsin-like (Pr2) cuticle-degrading proteases were produced and the enzyme kinetic properties showed better activity of Pr1 in comparison with Pr2. The proteases were purified using acetone precipitation, Sephadex G-100 gel filtration and CM-Sepharose ion exchange chromatography, with a 5.09-fold increase in specific activity and 21.86% recovery. The enzyme molecular weight was estimated to be 47 kDa and the optimal pH and temperature were 8 and 45ºC, respectively. The purified protease was activated by divalent cations, Ca^{2} and Mg^{2}, and inhibited by NaCl, KCl and determined as a serine protease by inhibition of its activity due to using PMSF, EDTA, mercaptoethanol and SDS. Studies on the timing of the protease secretion in the presence of cuticular substrates could provide information about the role of the accumulated hydrolytic enzymes during pathogenesis to better understand these processes.

Keywords: *Beauveria bassiana*, Proteases, *Eurygaster integriceps*, Cuticle

Poster / Fungi Wednesday, 16:30 F-03

Fungi related to larvae of red palm weevil *Rhynchophorous ferrugineus* (Coleoptera: Curculionidae)

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As a first step to developing effective biocontrol strategies, we analyzed the pathogens and mainly the entomopathogenic fungal species, present into dead larvae of *Rhynchophorous ferrugineus;* a quarantine pest recently introduced and acclimated in Sicily (Italy). From 2008 to 2010, we estimated the natural larval mortality (> second instar) of *R. ferrugineus* collected from infested palm *Phoenix canariensis;* that was variable from 10 to 35%, according to the different seasons Among the different pathogens recorded the fungi seems have an important role, than in order to distinguish saprophytic or opportunistic species from potential pathogens (including the non cultivable ones) we applied different protocols based on observation by optical and scanning electron microscopy and on molecular analysis. Among fungi species, *Acremonium* and *Beauveria* were identified as agents of eumycotic white grain mycetoma, found into several dead larvae. Both of them are considered for biological control and *Acremonium* is reported to be endophytic with a potential role in the reducing population of phytophagous. The identified species should be play a potential role in controlling *R. ferrugineus* population in Sicily.

Keywords: Enthomopatogenic fungi, biological control, palm trees

Poster / Fungi Wednesday, 16:30 F-04

Combination of entomopathogenic fungi *Beauveria bassiana* (Bals.) and *Lecanicillium muscarium* (Petch.) with insecticide imidacloprid on different nymphal instars of greenhouses whitefly *Trialeurodes vaporariorum* West. (Hemiptera: Aleyrodidae) in laboratory conditions

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In this study, the compatibility of the entomopathogenic fungi *Lecanicillium muscarium* and *B. bassiana* with imidacloprid to control the different nymphal instars of the greenhouse whitefly, *Trialeurodes vaporariorum*, was investigated. Whitefly-infested tomatoes were sprayed with imidacloprid and after 8 days different concentrations of fungi were applied. The results showed that the mortality rate of nymphs was significantly higher when a combination of imidacloprid and *B. bassiana* was used compared to *B. bassiana* used alone. Furthermore, mortality was higher when *L. muscarium* was applied on different nymphs of *T. vaporariorum* compared to *B. bassiana*. In controlling of the young nymphs, when a combination of imidacloprid and *L. muscarium* was used, the insecticide had no negative effect on the germination of conidia and growth of mycelium of *L. muscarium*. To control the old nymphs of *T. vaporariorum*, the higher concentration of imidacloprid and *L. muscarium* used together caused a significantly higher mortality percentage in comparison to imidacloprid or *L. muscarium* used alone.

Keywords: Entomopathogenic fungi, Imidacloprid, *Lecanicillium muscarium*, *Beauveria bassiana*, *Trialeurodes vaporariorum*

Poster / Fungi Wednesday, 16:30 F-05

The effect of selected plant volatiles on conidial germination of aphid-pathogenic fungi

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Studies of tritrophic interactions aim to understand the often complex relationships between plants, herbivores and natural enemies. The aim of this work was to determine the influence of the volatile compounds emitted by primary host of *Rhopalosiphum padi* on conidial germination of aphid-pathogenic fungi *Pandora neoaphidis*, *Conidiobolus obscurus* and *Lecanicillium muscarium* in an olfactometer study. The volatiles were trapped in the field during the aphid spring migration and were identified by HS-SPME/GC-(EI)MS method. The emitted volatiles were as follows: (Z)-3-hexen-1-ol, benzaldehyde, benzoic alcohol, methyl salicylate, farnesene. The experiment on spore germination was carried out on slides with water-agar medium placed in oflactometer. The results showed that the influence of volatile substances on the process of spore germination varied and depended both on the fungal species and type of test compound. Occurring during the spring development of the population of oligophagous aphid species cis-3-hexen-1-ol, stimulated conidial germination of the fungus *C. obscurus*, but inhibited this process in case of *P. neoaphidis* and *L. muscarium*. Intensely emitted by black cherry plants during the spring migration of aphids - methyl salicylate - limited conidial germination of *C. obscurus* and *P. neoaphidis* but slightly stimulated spore germination of *L. muscarium*. Benzaldehyde and benzoic alcohol, which are strongly emitted by the primary host during the autumn re-emigration of aphids, only marginally inhibited conidial germination of fungi. A mixture of three volatile compounds (cis-3-hexene-1-ol + benzaldehyde + benzoic alcohol) stimulated germination of two entomophthoralean fungal species, whereas in the case of the fungus *L. muscarium* its impact was negligible. Conducted experiment has shown that the spores of entomophthoralean fungi are more sensitive to volatile substances emitted by the primary hosts of aphids than spores of *L. muscarium.*
This work was supported by the Ministry Science and Higher Education, project
No N310 003 31/0264

Keywords: Plant volatiles, aphids, entomopathogenic fungi, spore germination

Poster / Fungi Wednesday, 16:30 F-06

Horizontal transmission of *Lecanicillium* spp. from infected cadaver of cotton aphid to healthy population

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Infection spread of *Lecanicillium* spp. basically occurs via contagious infection, and some fungal primary traits would affect to them. In our previous study, three strains of *Lecanicillium* spp. (Vertalec, Mycotal; commercialized strains and 2aF43; hybrid strain) showed different mode of mycelial growth and formation of conidial heads on aphid cadaver. Vertalec showed short mycelial growth with a lot of outward conidial heads, Mycotal showed abundant mycelial growth with a few outward conidial heads, and 2aF43 showed abundant mycelial growth with a lot of outward conidial heads. To evaluate the horizontal transmission efficacy, one or ten infected aphid cadavers were placed on the leaf disks and incubated for 5 or 7 days. After that, ten fresh aphids were released to these leaf disks for 2 days, and they were removed to new assay chamber to evaluate the infection rate in healthy populations. The infectivity from 5 days incubated one aphid cadaver on leaf disk resulted in equivalent degree in Vertalec and 2aF43, but there were no differences on 7 days incubated leaf disks among three strains. Horizontal transmission efficacy of 5 days incubated ten aphid cadavers on leaf disks showed that 2aF43 was higher infectivity than the others, but in 7 days incubated leaf disks revealed same degree in Vertalec and 2aF43. These results shows the mycelial growth and the mode of formation of conidial heads were important factor for horizontal transmission of *Lecanicillium* spp. It seems that there is different strategies for spread of infection among aphid population.

Keywords: Biological control, cotton aphid, *Lecanicillium* spp.

Poster / Fungi Wednesday, 16:30 F-07

Virulence of the entomopathogenic fungi isolated from the Great Spruce Bark Beetle, *Dendroctonus micans* (Kugelann) (Coleoptera: Scolytidae)

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The great spruce bark beetle, *Dendroctonus micans* (Kugelann) (Coleoptera: Scolytidae), has been a potential threat for not only Turkey, but also the entire Eurasian spruce forests for many years. Control strategies which have been applied so far are still insufficient to prevent its damage. A total of 12 fungal strains including *Lecanicillium muscarium* (Petch) Zare and Gams, *Isaria farinosa* (Holmsk.: Fr.) Fr., *Fusarium* sp., *Beauveria bassiana* Sensu Lato and *Beauveria* sp. were isolated from larvae and adults of *D. micans* collected from oriental spruce stands in the vicinity of Trabzon, a city of Turkey. Among all isolates, *Lecanicillium muscarium, Isaria farinose* and *Fusarium* sp. were the first time isolated from this pest. A 1× 106 ml-1 spore suspension was applied to larvae and adults of the pest for virulence test. The highest mortality and mycosis value for larvae were obtained from the isolate Dm-5 (*Beauveria bassiana*) with 90% mortality within 10 days after application and 90% mycosis value ($p<0.05$). Dm-5 produced also 93% both mortality and mycosis value on adults at the same period. On the other hand, the highest mortality and mycosis value for adults were obtained with isolate Dm-6 (*Beauveria* sp.), producing 100% mortality within 10 days after application and 80% mycosis value ($p<0.05$). These results indicate that isolate Dm-5 and Dm6 seem to be promising fungal biocontrol agents against *D. micans*, a serious pest of spruce forests.

Keywords: *Dendroctonus micans*, Entomopathogenic fungi, *Beauveria bassiana*, Microbial control

Poster / Fungi Wednesday, 16:30 F-08

CTC medium: a novel dodine-free selective medium for isolating entomopathogenic fungi, especially *Metarhizium acridum*, from soil

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The selective media most commonly used for isolating hyphomycetous species of entomopathogenic fungi from non-sterile substrates rely on *N*-dodecylguanidine monoacetate (dodine) as the selective fungicide. Although this medium is effective for isolating many species of *Metarhizium* and *Beauveria* from soil; it is an inefficient medium for isolation of an important *Metarhizium* species, *M. acridum*, from non-sterile soil. The current study was directed to formulating a dodine-free selective medium that is efficient for isolating naturally occurring *Beauveria* spp. and *Metarhizium* spp., especially *M. acridum*, from soil. The selective medium (designated CTC medium) consists of potato dextrose agar plus yeast extract (PDAY) supplemented with chloramphenicol, thiabendazole and cycloheximide. In comparisons with selective media previously reported in the literature, the CTC medium colonies that were afforded larger and had both earlier and more abundant conidiation of entomopathogenic fungi, features which greatly facilitated identification of the emerging entomopathogenic fungi. In addition to efficient re-isolation of *M. acridum*, this medium also is an effective tool for selective isolation of *M. brunneum*, *M. robertsii*, *B. bassiana* and *B. brongniartii* from non-sterile field-collected soil samples inoculated (spiked) with fresh conidia in the laboratory.

Keywords: Selective medium, entomopathogenic fungi, *Beauveria bassiana*, *Beauveria brongniartii*, *Metarhizium brunneum*, *Metarhizium acridum*, chloramphenicol, thiabendazole, cycloheximide

Poster / Fungi Wednesday, 16:30 F-09

Both alpha and beta tubulins are involved in *Metarhizium anisopliae* resistance to carbendazim

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Metarhizium anisopliae resistance to fungicide sprays often encountered in the field is important for the success of mycoinsecticde application in insect control. In this study, 30 *M. anisopliae* stains were assessed for their resistance to carbendazim (methyl 2-benzimidazole carbamate; MBC) and only one of them showed low resistance to the chemical. This strain, namely Ma 456, was subjected to the chemical pressure for mutation, yielding 11 mutants with the resistance enhanced from 8.8 to 376 fold. The sequences of their α-tubulin and β-tubulin were then compared with those of the wild-type stain. As a result, all the mutants were found sharing a common mutation of α-tubulin S231L and/or β-tubulin S144G, an indication for the importance of both S231 and S144 sites in the fungal MBC resistance. Other sporadic mutation occurred at more site of α-tubulin than β-tubulin. The increased MBC resistance was accompanied by the decrease of conidial LT_{50} (tolerance to 48°C) from 31.4 min (Ma 456) to 10.5-29.1 min (mutants) and a change of conidial UV-B LD_{50} (tolerance to UV-B irradiation) from 0.19 J/cm^2 (Ma 456) to 0.08-0.23 J/cm^2 (mutants). This is the first report on the involvement of both α- tubulin and β-tubulin in the MBC resistance of *M. anisopliae*.

Keywords: *Metarhizium anisopliae*, tubulins, carbendazim, resistance

Poster / Fungi Wednesday, 16:30 F-10

Influence of host plant species on Diamond Back Moth, *Plutella xylostella* susceptibility to *Beauveria bassiana* and *Metarhizium anisopliae* and on its fertility life table parameters

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Diamondback moth (DBM), *Plutella xylostella* (L.)(Lepidoptera: Plutellidae) is still one of the most destructive insect pests of cruciferous crops throughout the world. We investigated the influence of host plant species including canola (*Brassica rapa* cv. Okapi), cauliflower (*Brassica oleracea* var. Arizona) and chinensis cabbage (*Brassica pekinensis*) on DBM susceptibility to *Metarhizium anisopliae* and *Beauveria bassiana* and on its fertility life table parameters at the laboratory conditions. Virulence of different isolates of *M. anisopliae* and *B. bassiana* were carried out against the larvae of DBM in laboratory. Our results indicated that all these different isolates of *M. anisopliae* and *B. bassiana*, were pathogenic to the DBM. However, there was a variation in their virulence, ranging 20.7 to 83.3% total mortality at seven days monitoring. Although the highest mortality (71.7 %) in DBM larvae with *B.bassiana* treatment was noted on canola and with *M. anisopliae* treatment (83.27%) was seen on cauliflower, but analysis of variance indicated that these diferences were not significant. The duration of each life stage, longevity, reproduction rate, the intrinsic rate of natural increase (r_m), net reproductive rate (R_0), mean generation time (T), doubling time (DT) and finite rate of increase (λ) of the DBM were calculated on these three different host plants. Differences in fertility life table parameters of DBM were analyzed among host plants by the Birch and Jackknife methods. Lower larval development time (7.16 days), higher reproduction (215.4±2 eggs) and higher r_m (0.246±0.001) were recorded on chinensis cabbage, whereas these related values were 11.8 days, 159.6±0.05 eggs and 0.174 on canola, repectively.

Keywords: Host plant species, fertility life table parameter, *Plutella xylostella*, *Metarhizium anisopliae*, *Beauveria bassiana*

Poster / Fungi Wednesday, 16:30 F-11

Molecular Characterization and Virulence of *Beauveria* spp. from the Pine Processionary Moth, *Thaumetopoea pityocampa* (Lepidoptera: Thaumetopoeidae)

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The pine processionary moth *Thaumetopoea pityocampa* (Den. and Schiff.) is one of the most harmful pests for pine species in Mediterranean countries including Turkey. Caterpillars of *T. pityocampa* are not only significantly harmful to forest trees but also responsible for various allergic reactions in humans and animals. In this study, in order to find a more effective and safe biological control agent against *T. pityocampa*, we investigated fungal pathogens of *T. pityocampa* collected from different populations of forests of Black Sea Region of Turkey from 2005 to 2007 and tested their insecticidal activity on it. Five different fungal strains were isolated and identified based on their morphological and molecular characteristics including ITS and partial sequence of EF1-α. Based on these characteristics, four isolates were identified as *Beauveria* cf. *bassiana* and one isolate was identified as *Beauveria bassiana*. Among these isolates, *B. bassiana* KTU-24, *B.* cf. *bassiana* KTU-66 and KTU-67 showed the highest virulence with 100% mortality within 10 days after application. *B. bassiana* isolate KTU-24 produced the same rate of mycosis with mortality value. Consequently, *B.* cf. *bassiana* seems to be a natural pathogen of *T. pityocampa* and isolate KTU-24 can be utilized as a possible biological control agent against *T. pityocampa*.

Keywords: The pine processionary moth, fungal pathogen, *Beauveria* spp., microbial control

Poster / Fungi Wednesday, 16:30 F-12

New insights into biocontrol of the white grub, *Polyphylla olivieri*

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White grub, *Polyphylla olivieri* (Col., Melolonthidae) is a serious pest of fruit and other trees. The larvae damage the roots and cause trees to die and ultimately fall. This scarabeid is a dangerous pest of orchard trees in most parts of Iran. It damage are severe especaially for youg trees. Complex life cycle of this pest and difficulty in application of synthetic pesticides for its control are from most obvious reason to evaluate environmental friend-techniques including application of natural insect pathogns like *Metarhizium anisopliae*. Control measures for white grubs have depended mainly on conventional chemical pesticides. While alternate control methods of this group is possible with entomopathogens, but use of these technologies is limited. Currently, there are some products formulated with fungal pathogens of insects that using commercially for controlling white grubs through the world. In this research, potential of a powerfull insect pathogen was investigated whether might be useful for control of the white grub, *Polyphylla olivieri.* Exploratory survey revealed that *M. anisopliae* is widely distributed in the Tehran soils province. Disease prevalence of *M. anisopliae* in grub cadaver was between 0 and 2% depending on host origin and species. Analysis of soils from different regions showed that *M. anisopliae* is common and was present in about 10% of the samples irrespective of their origin. Screening of fungus isolates in time mortality studies indicated that two isolates gave over 60% infected grubs. Although some of the isolates of *M. anisopliae* were highly pathogenic more study needed about their compatiblity with the pesticides, strain improvement to enhance epizootic potential may be appropriate. *M. anisopliae* has the potential to be an effective biological control agent of *Polyphylla olivieri.*

Keywords: *Polyphylla olivieri*, *Metarhizium ansiopliae*, Iran

Poster / Fungi Wednesday, 16:30 F-13

First evaluation of *Beauveria bassiana* against the *Gyropsylla spegazziniana* Lizer and Trelles (Hemiptera: Psilidae) on Paraguay tea (*Ilex paraguariensis* St. Hil.) leaves

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Gyropsyllaspegazziniana (G.s.) is one of the most important pests of Paraguay tea crops in all South America (Brazil, Argentina and Paraguay). Natural enemies have been registered on *G. spegazziniana*, including entomopathogen fungi *Zoophthora radicans*. Here, we report the first evaluation of *Beauveria bassiana* pathogenicity over this pest on laboratory. Branches from Paraguay tea plants with G.s. galls were maintained in glass Erlenmeyer with waterin organza cloth cages. Each treatment consisted of Paraguay tea branches infested with 15 to 30 recent emerged nymphs/leaf (3 replicatins/treatment). The fungus suspension (1×10^9 conidia/mL) was sprayed on the leaves that were transferred to cages (26 ± 1 °C, 70 ± 10% RH and 14 h photophase). Daily evaluation showed after five days, high level of insecticidal activity. The pathogenicity was confirmed by visual observation of fungus sporulation on insect cadavers. The total mortality ranged 25 to 65% and the higher confirmed mortality was 35% due to Unioeste 52 strain (obtained from the adults of the lesser mealworm, *Alphitobius diaperinus*). There is potential of the fungus to control this pest, although complementary studies should be realized.

Keywords: Entomopathogenic fungi, biological control, pathogenicity

Poster / Fungi Wednesday, 16:30 F-14

***Evlachovaea*: Coming home to say goodbye!**

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Evlachovaea kintrischica Borisov and Tarasov (1999, Mikol. Fitopatol. 33: 248-256) from southwestern Georgia, just 200 km from this meeting site, was described as the sole species from a new genus distinguished by an unusual mode of conidiogenesis. Since that time, a number of fungi have been found from a wide range of insect hosts and geographical sites that have a similar mode of conidiogenesis (in which there is a zipper-like alternation of the orientation of conidia in ribbon-like conidial chains), and even a recognition that some of these conidial fungi are the anamorphs of *Cordyceps* teleomorphs. The results of molecular studies of a range of these *Evlachovaea*-like fungi from central Brazil and other locations around the world confirmed that (1) this unusual mode of conidiogenesis is not unique to a single monophyletic group of fungi, (2) that *E. kintrischica* is insufficiently distinguished morphologically or genomically from *Isaria amoenerosea* and *I. cateniannulata* and, after more study, will have to be synonymized with one of these taxa, (3) and, accordingly, that the genus *Evlachovaea* must be treated as a synonym of *Isaria* in the family Cordycipitaceae, and (4) that there has been a history of problems with the formal identifications to species of *Isaria* and *Paecilomyces* that are being progressively addressed as the molecularly based taxonomic data about these important entomopathogens become more numerous within species and cover an increasing proportion of species in these genera.

Keywords: Hypocreales, Cordycipitaceae, Clavicipitaceae, systematics

Poster / Fungi Wednesday, 16:30 F-15

Longevity and fertility life table parameters of *Phytoseiulus persimilis* (Acari: Phytoseiidae) fed on untreated and *Beauveria bassiana* treated adults of *Tetranychus urticae* (Acari: Tetranychidae)

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The effect of feeding on either untreated or treated adults of the spider mite, *Tetranychus urticae* Koch by *Beauveria bassiana* DEBI008 at four time intervals was investigated to compare the longevity and fertility life table parameters of the predatory mite, *Phytoseiulus persimilis* at 25±1˚C, 60-70٪ RH and a photoperiod of 16:8 h (L: D). Data analysis showed that longevity of predatory mite fed on untreated mites (control) and treated mites (time interval 0) was higher in comparison with other time intervals of inoculation. Related results indicated that entomopathogenic fungus has unfavorable effect on predatory mite longevity. Our results showed that mean generation time (T), doubling time (DT), net reproductive rate (R_0), finite rate of increase (λ) and intrinsic rate of natural increase (r_m) for predatory mite are strongly affected with fungus presence and these parameters had significant differences among our treatments. The least r_m value was observed in the time interval of 72 hours post-inoculation. More details in effect of *Beauveria bassiana* on the fitness and quality of *T. urticae* and consequently decreasing the longevity and intrinsic rate of *P. persimilis* will be presented.

Keywords: Fertility life table parameter, *Beauveria bassiana*, *Tetranychus urticae*, *Phytoseiulus persimilis*, longevity

Poster / Fungi Wednesday, 16:30 F-16

Preliminary results on the occurence of pathogens in the Pine shoot beetles *Tomicus piniperda* (L) (Coleoptera, Scolytidae) in Georgia

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The Pine shoot beetles *Tomicus piniperda* was occur on Pinus *Sosnovsky* in the pine forest of Easter Georgia. Adult beetles were collected three location of pine forest in 2009.

Single findings of the entomopathogenic fungus *Beauveria bassiana* have been detected in the population of *T. piniperda*. Isolated fungus were cultivated on PDA media for 12-15 days at 25 ^{o} C and were tested to adult beetles of this pest. Healthy beetles were collected by hand from infested trees or cutting infested log section from pine trees and placed on pine-bark pieces (10x15 cm) treated with of fresh cultural suspension of *B. bassiana* (1.0 X 10^6 and 1.0 X 10^7). The beetles of each variant were placed in the container and incubated at 25 ^{o}C (±3 ^{o}C), without light and at ~90% RH. Death beetles were removed daily and replaced on the Petri dishes for the conidia develop. The experiments showed that isolated *B. bassiana* is high virulence for the adults *T. piniperda*, and mortality was 81.5 - 100 %, in less then 8 day.
In our study one microsporidium infection was found in the midgaut overwinter adults of *T. piniperda* . Smears were examined at the normal microscope at x 300 – 600 magnification. The most probably it is *Canniga sp*. Furthermore, parasitic larvae of nematodes were found in haemolymph of beetles.

Keywords: *Tomicus piniperda*, *Beauveria bassiana*, *Canniga* sp.

Poster / Fungi Wednesday, 16:30 F-17

Evaluation of *Isaria fumosorosea* CCM 8367 for the control of *Cameraria ohridella*, and effects on beneficial parasitoids

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The horse chestnut leaf-miner *Cameraria ohridella* is an invasive pest which has spread rapidly through Europe over the last two decades. The aim of the present study was to test the effect of *Isaria fumosorosea* CCM 8367 on this pest and its parasitoids. Dry horse chestnut leaves (200 g per replication) with hibernating *C. ohridella* pupae were immersed for five minutes into the suspension of blastospores at concentration 5 × 107 spores/ml while control was immersed into distilled water only. The leaves were than placed into photoeclectors and numbers of *C. ohridella* adults and hymenopteran parasitoids emerging from the leaves were recorded. In addition, samples of leaves were dissected to determine mortality of *C. ohridella* pupae. Both the control and the treatment were replicated ten times. The experiments were carried out in a climatized chamber at 23˚C and 16L:8D photoperiod. The results revealed that the mean total number of *C. ohridella* adults emerged until the 18th day from the control and treatment was 637.0 and 100.9, respectively. The difference was highly statistically significant. Mortality of pupae in the control leaves sampled 10, 12 and 14 days after the treatment was 10.0, 13.3 and 16.7%, respectively while in *I. fumosorosea* treated leaves it was 76.7, 86.7 and 86.7%, respectively. The mean total number of parasitoids was significantly reduced in the fungus treatment (21.0) compared to the control (96.3). We can conclude that *I. fumosorosea* CCM 8367 is perspective for *C. ohridella* control but the impact on its natural enemies should come into question. Supported by MSMT grant 2B06005.

Keywords: Horse chestnut, Gracillariidae, leaf-miner, natural enemies

Poster / Fungi Wednesday, 16:30 F-18

Entomopathogenic fungus of *Coccinella septempunctata* L. (Col.: Coccinellidae) in the Uludaz Hill of the Cimen Mountain, Kahramanmaras

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Seven spotted lady beetle, *Coccinella septempunctata* L., an aphidophagus coccinellid, has very wide geographical distribution. This species shows univoltinism in Turkey. Some of individuals can hibernate near breeding sites and others migrate to prominent hills or to the top of mountains. In Kahramanmaras Province, *C. septempunctata* adults migrate about early June to Uludaz Hill of the Cimen Mountain where spend summer, fall and winter. Thousands of individuals hide under the rocks or prickly thrift, *Acantholimon* sp.. The main purpose of the study was to observe mortality factors of *C. septempunctata* at high elevation. Samples of alive and dead *C. septempunctata* were taken monthly for two years (2008, 2009) between June to November. The entomopathogenic fungi were prepared on SMY for comparison *in vitro* growth and examined under light and scanning electron microscope for detailed characterization. Since many individual hibernated together, entomopathogenic fungus *Beauveria bassiana* were important mortality agent. The only parasitoid found in ladybeetles was *Dinacampus coccinellae* Schrank (Hymenoptera: Braconidae).

Keywords: *Coccinella septempunctata*, entomopathogenic fungi, *Beauveria bassiana*

Poster / Fungi Wednesday, 16:30 F-19

Study of entomopathogenic fungi Aschersonia isolates from Chinese citrus orchards on pathogenicity, phylogeny and genetic diversity

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Fifty-nine *Aschersonia* isolates were collected from citrus orchards, main cultivated areas of citrus in China. They were analyzed of their pathogenic potential to *Dialeurodes citri* (Ashmead) larvae, their phylogeny based on PRB1 sequence analysis and their genetic diversity evaluated by Inter-Simple Sequence Repeats (ISSR) technique. The pathogenicity of the naturally occurring entomopathogenic fungus against the citrus whitefly was tested under glasshouse conditions. Of the 59 isolates tested, four resulted in mortality rates ≥50%. Isolates YW3 that had the best performance caused an accumulative mortality of 67.5 ± 5.1% within 2 weeks on the 1×10^6 spores/ml concentration. Phylogenetic analyses of partial sequences from RNA polymerase subunit 1 (RPB1), were conducted to determine the relationships of the

aschersonia isolates to other aschersonia species. The sequences were aligned using ClustalW and aligned sequences from RPB1 gene included 663 characters. Phylogenetic analyses show that aschersonia WDC2 belongs to a strongly supported clade that include *Aschersonia placenta*, whereas other isolates belong to another group that include *Aschersonia aleyrodis* . Twelve primers were chosen in ISSR markers for reproducibility and high polymorphism analysis. A total of 114 fragments were amplified in all 59 isolates, among these, 108 (94.7%) were polymorphic. The genetic similarity values among aschersonia isolates ranged from 0.456 to 0.973. The dendrogram created from UPGMA analysis based on Nei and Li's coefficient showed that 59 aschersonia isolated could be divided into two clusters. The results also indicated that there was a certain association between Aschersonia isolates and their geographical origins.

Keywords: Aschersonia, pathogenicity, phylogeny,Genetic diversity, ISSR

Poster / Fungi Wednesday, 16:30 F-20

The melanin produced by recombinant *Escherichia coli* enhances the survival of *Beauveria bassiana* conidia under UV-B irradiation

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Solar UV is well known to affect conidial viability of fungal biocontrol agents exposed to outdoor conditions and such effect can be mitigated by adding melanin to fungal formulation. A gene (*BcTyr*) encoding *Bacillus cereus* tyrosinase that enables to convert tyrosine to melanin was cloned from the bacterial genomic DNA and expressed well in *Escherichia coli* transformed with a recombinant plasmid pET29b-BcTyr. The expressed BcTyr in the tyrosine-inclusive culture of a selected transgenic strain incubated to log-growth phase at 37°C was exploited to induce melanin production at 30°C by adding 0.5 mM isopropyl-b-D-thio- galactopyranoside (IPTG) to the culture. Crude melanin extract was obtained by precipitation under an acid condition. The melanin extract was found enhancing ~25% survival of *Beauveria bassiana* conidia exposed to the UV-B irradiation of 0.8 J/cm^2. This indicates a high potential for the melanin to be used a UV protectant in the fungal formulations against arthropod pests.

Keywords: *Beauveria bassiana*, melanin, UV-radiation

Poster / Fungi Wednesday, 16:30 F-21

Pathogenicity of selected entomopathogenic fungi to larvae of *Spodoptera littoralis* (Boisd.) (Lep.: Noctuidae) in laboratory conditions

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Spodoptera littoralis is a poliphagous pest of several crops and its management basically depends on chemical insecticides where population level is troublesome. Entomopathogenic fungi have been considered as alternative control agents. In this study six fungus isolates (*Beauveria bassiana* 1512, 3288, 6646; *Metarhizium anisopliae* 2735, 3293; *Iseria tenuipes* 2488), obtained from ARSEF, were tested against second instar larvae by dipping technique. All tests were performed at 25±2°C in darkness. RH was maintained at 100% in the first 24 hours and thereafter at 65±5%. All the tests had three replicates with 10 larvae in each unit. Control received only 0.02% Tween 80, which was also used to prepare conidiospore concentrations. In the first test, larvae were challenged by a single concentration ($1x10^8$ conidiospores ml^{-1}) of all the isolates. Mortality of the larvae varied between 20-87%. Five successive concentrations ($1x10^4$ – $1x10^8$ conidiospores ml^{-1}) of 3293, 2488 and 6646, which gave the highest mortality in the first test, were used in the second test to evaluate their pathogenicity in detail. 6646 gave the lowest LC_{50} of $8.4x10^6$ conidiospores ml^{-1}. The third test was performed to find the time-mortality relations of 3293 and 6646. 2488 was eliminated due to long time taken to kill in the previous test. Expected LT_{50} value was calculated as 4.27 days for 6646. It is concluded that *B. bassiana* 6646 had the highest pathogenicity amongst the tested isolates under laboratory conditions. Its evaluation for the control of the pest requires further tests, especially those towards to use in field conditions.

Keywords: Microbial control, biological control, *Beauveria bassiana*, *Metarhizium anisopliae*, *Iseria tenuipes*

Poster / Fungi Wednesday, 16:30 F-22

Endophytic colonization of entomopathogenic fungi in strawberry plants

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Lygus bug is a major pest of strawberries in coastal California. Currently registered chemicals provide limited control especially for adult bugs. *Beauveria bassiana* and *Metarhizium anisopliae* are pathogenic to Lygus. Commercial formulations of *B. bassiana* are registered for use on strawberries, but information on their field efficacy in California is lacking. This research is a part of a major study evaluating entomopathogenic fungi for Lygus control. In a greenhouse study, strawberry transplants were inoculated with commercial and indigenous isolates of *B. bassiana* (GHA and a California isolate, SfBb1) and *M. anisopliae* (F52 and a California isolate, GmMa1) using three inoculation procedures – mixing $1X10^7$ conidia per gram of vermiculite, dipping roots of the transplants in $1X10^7$ conidia/ml conidial suspension, and pouring 100 ml of $1X10^7$ conidia/ml suspension at the base of the potted transplant. After 6 weeks, some plants were reinoculated with $1X10^9$, $1X10^{10}$ or $1X10^{11}$ per plant by pouring a 200 ml suspension at the base of the plant. Colonization of the roots, petioles and leaf lamina by the two fungi was monitored 1, 3 and 6 weeks after both inoculations. Results of the colonization and its persistence in strawberry plants will be presented.

Keywords: Beauveria bassiana, Metarhizium anisopliae, Lygus, endophyte

Poster / Fungi Wednesday, 16:30 F-23

Perspectives of the Colorado potato beetle fungi pathology in Georgia

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The Colorado potato beetle (CPB) – *Leptinotarsa decemlineata* Say is the most economically damaging pest to potatoes in most areas of Georgia. The beetle may also feed on tomato, eggplant, tobacco, pepper and etc. CPB has developed resistance to most registered pesticides, making it one of the most difficult insect pests to control. It is very important the plant protection from CPB by using of safe for man and environment. Entomopathogenic fungi (EPF) are considered as the main natural agents for biological control. As the results of researches the CPB populations distributed in East and West Georgia the mortality (1-2%) of CPB larvae and imago has been caused by the EPF *Beauveria bassiana.* This naturally occurring organism is considered as a local strain for further investigations. Along with this the different strains of *B. bassiana* LRC_{107}, T_3, T_4, T_5, T_6 (introduced under the *MTA* agreement Lethbridge Research Centre, Agriculture and Agri-Food Canada and Kanchaveli L. Institute of Plant Protection) were studied toward CPB larvae and imago. The isolates were cultured on potato dextrose agar (PDA) for 14-20 days at 23-24^{o}C. The conidia suspensions were filtered through a sterilized cheese-cloth and enumerated using a haemocyrtometer. A 2.7 X 10^6 per ml suspension was used for treatment of CPB different instars larvae and imago. The control being treated with commercial mycopesticide *BotaniGard ES* - 0.5%. Statistical analysis of each bioassay was conducted according to two-way ANOVA (GrafPadPrism5). The strains T_3, T_4 and T_5 differ high indexes of mortality (60-70%) and not lagging behind the control.

Keywords: The Colorado potato beetle, Entomopathogenic fungi, Plant protection

Poster / Fungi Wednesday, 16:30 F-24

Characterization of three different catalase genes in entomopathogenic fungus *Beauveria bassiana*

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Catalases in cellular antioxidant responses may play important roles in the detoxification of hydrogen peroxide. In this study, three catalases were identified from *Beauveria bassiana* by means of non-denaturing polyacrylamide gel electrophoresis and staining for catalase activity. Two monofunctional catalase genes (*BbCatA* and *BbCatB*), and one bifunctional peroxisomal catalase gene (*BbCatP*) were cloned from the fungal genome. The *BbCatA* gene was deduced encoding a 724-aa polypeptide (80.7 kDa) sharing ~70% sequence identity with the catalases of *Gibberella moniliformis* and *Cochliobolus heterostrophus*. The deduced BbCatB (a 748-aa polypeptide, 81.6 kDa) shared 60-64% identity with the catalases of *Metarhizium anisopliae* and *Botryotinia fuckeliana*. However, BbCatA and BbCatB showed little homology in sequence and fell in the family of large-subunit catalases. The *BbCatP* gene consisting of a 2289-bp ORF and three introns was deduced coding a 762-aa catalase subunit. This deduced protein (84.2 kDa) was characteristic of a peroxisome-targeting signal (SKPRL) in the C-terminus, the catalase-peroxidase bifunction and ~70% sequence identity to the same bifunctional enzymes of *Penicillium marneffei* and *Aspergillus terreus*. The three catalases existing in *B. bassiana* indicate that the fungal entomopathogen is highly capable of detoxifying intracellular hydrogen peroxide, warranting further studies.

Keywords: *Beauveria bassiana*,catalase,antioxidant

Poster / Fungi Wednesday, 16:30 F-25

Functional characterization of hydrophobins in the entomopathogeneic fungus *Metarhizium anisopliae*

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Hydrophobins are small, cysteine-rich, secreted proteins ubiquitously produced by filamentous fungi and are speculated to function in fungal growth and development, although this has been rigorously tested for a few species only. Here, we report cloning of three hydrophobin genes from the entomopathogenic fungus, *Metarhizium anisopliae* and functional characterization of mutant strains. One of these genes (*HYD1/ssgA*) encodes a Class I hydrophobin identified previously (St Leger et al. 1992). Two new genes (*HYD2 and HYD3*), encoding Class-II hydrophobins were identified. To examine function, we deleted all three, separately, from the *M. anisopliae* strain KTU-60 genome using *Agrobacterium tumefaciens* transformation protocols. Hydrophobin deletion strains were screened for alterations in developmental phenotypes including growth, sporulation, pigmentation, surface properties, and virulence. All deletion strains were reduced in ability to sporulate, showed increased radial growth, and lacked wild-type pigmentation. Deletion of either *HYD2* or *HYD3* genes caused reduced pathogenicity on *Galleria mellonella* larvae. All strains retained wild-type surface hydrophobicity, except for one of three purified *mhyd3* deletion strains. Expression patterns of each of the three genes varied with developmental stage of the fungus and with genetic background of the mutants. Results suggest that hydrophobins are involved in conidiation, virulence, pigmentation and hydrophobicity in *M. anisopliae.*

Keywords: *Metarhizium anisopliae*, hydrophobin, virulence, sporulation, hydrophobicity

Poster / Fungi Wednesday, 16:30 F-26-STU

Susceptibility of *Ceratitis capitata* to *Beauveria bassiana* isolates from the Moroccan endemic forests of Argania spinosa

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The Mediterranean fruit fly (medfly), *Ceratitis capitata*, is widely distributed around the World and is regarded as the most important pest of agricultural resources. In Morocco, the situation is even more complicated since the medfly survives in the Moroccan large endemic forests of *Argania spinosa* (800,000 hectares). These 21 millions Argan trees are regarded as the World main refuge of *Ceratitis capitata*. The medfly management relies only on the insecticide uses in Morocco. Besides the environmental, ecological and human health problems caused by pesticides, they were not able to reduce significantly important medfly infestations. Therefore, alternatives strategies must be developed urgently to control this important pest.

The entomopathogenic fungi may be potentially used as an alternative method to control the medfly development. The knowledge of medfly life cycle indicates that only prepupae, pupae and adults could be targeted with entomopathogenic fungi. The eggs and the larvae could not be infested directly with the biocontrol agents since the egg hatching and the larvae development are accomplished inside the fruit. The Spanish and Kenyan laboratories have found promising results when *Beauveria bassiana* and *Metarhizium anisopliae* isolates have been used against *C. capitata.* In this study, the *B. bassiana* have been isolated from the Moroccan argan forests, known as the world main refuge of *C. capitata* and have been used in bioassays against medfly pupae and adults.

Hundreds of Moroccan *B. bassiana* isolates were tested against medfly pupae in a preliminary laboratory screen and only fifteen isolates have been chosen for further bioassays to determine the % of pupae mortality, the LC_{50} for pupae, the LT_{50} and the Average Survival Time (AST) for adults. These isolates caused different percentage of pupae mortality. The majority of *B. bassiana* isolates showed higher percentage of pupa mortality reached 91,67% and significant reduction of adults' emergence. The percentage of pupae mortality increased significantly when *B. bassiana* were re-isolated from the infected pupae and used against the medfly pupae. The LC_{50} varied from 2.85×10^3 to 3.68×10^4 conidia ml^{-1}. Two isolates had the lowest LC_{50} and were the most pathogenic fungi to medfly pupae. These *B. bassiana* isolates caused different percentage of adults' mortality with a maximum of 91,67%. Seven isolates had an AST less than 7 days and a LT_{50} less than 7,8 days against medfly adults. Furthermore, more than 40 isolates were tolerant to temperature as high as 45°C. These data compared to published papers on other isolates showed clearly that at least ten Moroccan *B. bassiana* isolates could be used a potential biocontrol agent against *C. capitata*.

Keywords: Biological control, entomopathogenic fungi, *Beauveria* spp., pathogenicity, *C. capitata*

Poster / Fungi Wednesday, 16:30 F-27-STU

Isolation of anamorphic entomopathogenic fungi from wild mosquitoes collected in Japan and Burkina-Faso

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Basically, control of vector mosquitoes are conducted by the application of synthetic chemical insecticides, but developments of insecticide resistance have been reported. Therefore alternative approaches are required, and anamorphic entomopathogenic fungi is emerged as one of candidate for such approach. However little is known about ecological relationships between mosquito and entomopathogenic fungi in the wild state. Then, our research has been focused on the isolation of entomopathogenic fungi which infecting or adhering to wild mosquitoes to construct fungal culture database with property of infectivity and pathogenicity. First, we isolated anamorphic entomopathogenic fungi from mosquitoes collected in Japan. Human bait and CDC light trap were used for sampling wild mosquitoes. These samples were homogenized in the water, and plated on the selective medium. As a result, 2,579 fungal isolates were obtained from 738 *Aedes* spp. and 3 *Culex* spp. Among them, 117 fungal isolates were entomopathogenic fungi, including *Beauveria* spp., *Isaria* spp. and *Lecanicillium* spp. Secondly, we sampled malarial mosquito, *Anopheles gambiae* at malaria-endemic area, Burkina-Faso in West Africa. 13,080 fungal isolates were detected from 1,652 *An. gambiae*, and among them 78 anamorphic entomopathogenic fungi was *Lecanicillium* spp. These results reveal that at least some entomopathogenic fungi ride on the mosquitoes in natural state, but Burkina-Faso sample (0.6%) have a much lower rate of entomopathogenic fungi detection than Japan sample (4.5%). Furthermore, intriguingly, nematophagous fungus, *Pochonia suchlasporia* and *Simplicillium lamellicola* which closely related to entomogenous *Lecanicillium* spp. were also isolated from wild mosquitoes.

Keywords: Biological control, Burkina-Faso, fungi, Mosquitoes

Poster / Fungi Wednesday, 16:30 F-28-STU

Occurrence and genetic diversity of native entomopathogenic fungi from soils in Korea

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In agricultural fields, the entomopathogenic fungal species have been investigated for their potential as the biological control agents due to their role of natural enemies for insects. To address the

requirements of a potential South Korea based biocontrol effort using entomopathogenic fungi, we investigated the occurrence of various entomopathogenic fungi in 1080 soil samples representing from various area and locations in South Korea. Entomopathogenic fungi were isolated from soils using semiselective medium SDA-D50 contained saboraund dextrose agar, 50 mg/ml dodine 100 mg/ml chloramphenicol and 50 mg/ml streptomycin. The isolated putative fungi were identified by the determination of internal transcribed spacer (ITS) region sequences of the nuclear ribosomal analysis. As a result, entomopathogenic fungi were found to occur in 33.7% of the soil samples studied. The most abundant species were *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metschn.) Sorok. Isolates of *B. brongniartii, Cordyceps sp., Lecanicillium sp., Isaria sp. and Tolypocladium cylindrosporum* were also found. The occurrence of entomopathogenic fungi was analyzed by the area and soil types. These positive entomopathogenic fungi may have potential against variety pests in agriculture.

Keywords: Entomopathogenic fungi, distribution, ITS, semiselective medium

Poster / Fungi Wednesday, 14:00 F-29-STU

Pathogenicity of *Beauveria bassiana* and *Metarhizium anisopliae* to the *Tetranychus urticae* and *Metaseiulus occidental*

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The tow-spotted spider mite *Tetranychus urticae* (Acarina: Tetranichidae) is spread in the Georgia and feeds on more than 200 varieties of trees and herbaceous plant. *Metaseilius occidental* is predators from the family Phytosaiidae, which introduced from Canada. At present there is successful biological control agents against the tow –spotted spider mite.
Recent outbreak of *Tetranychus urticae* in Georgia promoted approaches to control the pest. Our aim of this study was to established a biocontrol strategy using the fungus. For this purpose, pathogenicity of *Beauveria bassiana* and *Metarhizium anisopliae* were evaluated in laboratory bioassays with *T. urticae* and *M. occidental* larvae. Fungal suspensions of *B. bassiana* and *M. anisopliae*, were prepared from 2 week-old cultures grown on PDA. Suspensions of the isolates were 8 $x10^6$ conidia /ml. 1ml suspension was applied on Munger cells and placed onto 2^{nd} and 3^{th} instars larvae of target mites. The cells were held in plastic bags to prevent desiccation and kept at 22^oC, with 16 / 8 light / dark regime.
Result showed that, insecticides activity of *B. bassiana* and *M. anisopliae* aginst *T. urticae* were higher and larvae mortality achieved 87-85%. However, fungi infected not only larvae of target insect, but having direct effect on the larvae of predators as well. *M. occidentalis* larvae mortality causing by *B. bassiana* was 57% and by *M. anisopliae* 70%. Both fungi are promising biological agents for the control *T. urticae.* Although, fungi have negative influence on the predators.

Keywords: *Tetranychus urticae, Metaseiulus occidental, Beauveria bassiana, Metarhizium anisopliae*

Poster / Fungi Wednesday, 16:30 F-30-STU

Interaction between *Lecanicillium* spp. and *Aphidius colemani* in biological control for *Aphis gossipii*

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It is known that some insect natural enemies transmit entomopathogenic fungi to pest insect population, and it brought synergistic interactions between pathogens and insect natural enemies would enhance pest control efficacy(Roy and Pell, 2000; Baverstock et al., 2010). In this experiment, we declare whether *Aphidius colemani* could be vector of *Lecanicillium* spp. for control of *Aphis gossipii.* Second and third instar lavae of *A. gossipii* were been treated with a spore suspension of *Lecanicillium* spp. on 0, 48 or 96h (sporulating cadaver) beforehand encountering to female *A. colemani*. As a result, *A. colemani* did not oviposited to sporulating cadaver. However, there were no significant difference among the mean oviposit rate of *A. colemani* on uninfected control, 0h and 48h infected aphids. So, *A.colemani* might contact with the fungal spores during host searching activities. Then, we evaluated *A.colemani* for the transmission ability of *Lecanicillium* spp. and observed the temporal aphid population changes. *A. colemani* treated with the spore suspension or untreated, put in the aphid population. *A. colemani* which treated with the spore suspention could be a vector of the fungi. In fungal treated plot, there were detected not only mummified aphid but also fungal infected aphid population. As a result, whole aphid population size was decreased by dual efficacy comparing with *A. colemani* only. These results suggested that *A. colemani* and *Lecanicillium* spp. could be used together to control *A. gossipii.*

Keywords: *Aphidius colemani*, transmission, *Lecanicillium* spp., biological control

Poster / Fungi Wednesday, 16:30 F-31

Paecilomyces lilacinus – from nematophagous fungus in field conditions to entomopathogenic fungus in greenhouse conditions

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Paecilomyces lilacinus is a typically soil-borne fungus. It is one of the most effective fungal species used in control of plant-parasitic nematodes and has also a good potential in control of insect pests. The Polish strain of *P. lilacinus* was isolated from eggs of

sugar beet cyst nematode (*Heterodera schachtii*) collected from fields on West part of Poland. The role of fungus in natural conditions on fields with sugar beet cultivation with modern technology (plant rotation and organic fertilizers) has been described. *P. lilacinus* was tested in laboratory, chamber and greenhouse conditions against root-knot nematodes (*Meloidogyne* spp.). Influence of nematode species, temperature, host plants on efficacy and spore production is described also.
The efficacy of fungus against greenhouse pests, like western flower thrips (*Frankliniella occidentalis*), red spider mite (*Tetranychus urticae*) and cotton aphid (*Aphis gossypii*) is discuss.

Key words: nematophagous fungi, *Paecilomyces lilacinus*, biological control

Thursday July 15, 2010
Osman Turan Congress Center

07:30 Bus pick up at hotels

Cross Divisional Symposium
Thursday, 8:00-10:00
Hasan Turan

Nematode and Fungus Divisions
Formulation of Entomopathogenic Fungi and Nematodes to Overcome Environmental Limitations
Organizer: Lawrence Lacey

Symposium Thursday, 8:00 182

Formulation and application of *Metarhizium acridum* for control of locusts: Overcoming the limitations of dry environments

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Applications of *Metarhizium acridum* remain the most important means of biological for Acridid pests, two isolates having been deployed as 'Green Muscle' (mostly in Africa) and 'Green Guard' (in Australia). Having been proven by staff of the International LUBILOSA Programme (now described in http://en.wikipedia.org/wiki/LUBILOSA) 'Green Muscle' is now available from commercial producers in Senegal and South Africa.
Conidia of *M. acridum* are formulated in appropriate oils, for ultra-low volume (ULV) rates of application: the conventional method for locust control. The concept relies on the use of highly virulent fungal isolates, but it may also be significant that fungal conidia are delivered as oil-based suspensions that are orders of magnitude more concentrated than most other mycoinsecticides delivery systems. An oil-miscible flowable concentrate (OF) formulation was developed, that is similar to use as conventional, chemical, locust control products. However, clients are increasingly requesting the dry spore (TC) formulation, since they value maximum storage stability more than ease of use in the field. The TC powder is produced to a strict specification and is easily suspended in standard spraying oils; the mixture can be left to stand overnight or longer if necessary.
We discuss how techniques can be applied to the development of other mycoinsecticides. Although emphasis has rightly been placed on host-pathogen relationships and environmental biology, success or failure also depends on product quality control and maximising the probability of encounter of a viable spore with the target pest. A key advance was the 'MycoHarvester' which efficiently separates conida from solid substrates; besides avoiding filter and nozzle blockages, this enables the development of stable, high quality formulations of predictable concentration.

Keywords: Formulation, Application, entomopathogenic fungi, *Metarhizium acridum*, Mycoharvester

Symposium Thursday, 08:30 183

Novel approaches in formulation of entomopathogenic fungi for control of insects in soil, foliar, and structural habitats: Thinking outside the box and expecting the unexpected

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By and large, mycoinsecticide formulations have involved sprayable products, typically oil flowables, emulsifiable suspensions, wettable powders, and water dispersable granules. Various nutritive or inert carriers have been used to create granular formulations for use against soil pests. Sometimes, however, completely different formulations, and applications of standard formulations arise from serendipity or necessity. Three examples are the keratin hydrolyzate foam formulation, an electrostatically-chargeable carnauba wax powder carrier, and *Metarhizium* microsclerotia-based granules discovered by USDA ARS in recent years. A foam formulation of Isaria fumosorosea blastospores was developed to fill the need for delivering the fungus into termite infested structures. The foam also has potential with tree bark treatments and even foliar applications. The carnuba wax dust carrier arose from the need to deliver fungal conidia to Varroa mite in bee hives. Development of the these three approaches will be discussed in this presentation. The discovery of *Metarhizium* microsclerotia was an unexpected result of developing a "standard" mycelial granule. The utility of granules made from these microsclerotia and their superiority to regular mycelial or other granules became readily apparent in production, shelf life, and bioassay studies.

Keywords: Formulations, Beauveria, Metarhizium, Isaria

Symposium Thursday, 9:00 184

Formulation and application: Key technologies to expand the use of entomopathogenic nematodes

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Entomopathogenic nematodes are used to control insect pests for about 25 years now and their use is slowly but surely extending from niche high value markets to large area crops including forestry. Tailored formulation and application solutions are needed for this expansion. In high value crops, the recommended nematode dose is high and often chosen arbitrarily. For instance, the dosage recommended in Europe for grass grub control is at least twice as high as the dosage recommended in the USA. In-depth studies in the critical application rate for specific crops are rare. One example for a method development to apply nematodes more efficiently is the use of *Heterorhabditis bacteriophora* against the Western corn root worm (*Diabrotica virgifera virgifera*). The

application technique needed to be adapted to the farmers needs. By developing a granular formulation which can be applied with the seeds, wasting nematodes between the rows can be avoided. The nematodes survive sufficiently long to efficiently reduce the number of emerging beetles. By using these granules, the application rate could be reduced from 0.5 million to 0.1 million nematode per m². Many foliar feeding insects, especially lepidopterans, are extremely susceptible to nematodes. Since nematodes are prone to desiccation on the leaves they need to be applied to the insect in a matrix which allows them to move and quickly penetrate into the insect‘s body. A suitable formulation was developed to control diamondback-moth larvae.
An under explored area for fungi and nematodes is their use in infection stations. Cockroaches can be effectively controlled with nematodes if contact with the nematodes is assured. Even arthropods which are commonly believed to be unsusceptible, like woodlice or adult weevils, get infected and killed in suitable infection stations.
Compared to fungi and bacteria, nematodes are characterised by a rather short shelf life. A substantial amount of time and money has been spent to overcome this limitation but with limited success. By enlarging the range of applications the demand of nematodes over the year will become more balanced and investing in production capacity is warranted. The recent investments in enlarging the scale of production have already resulted in a decrease in production costs. In synergy with more focused and parsimonious formulation and application methods this development can make nematodes economically compatible with chemical pesticides.

Keywords: Steinernema, Heterorhabditis, application technology

Symposium Thursday, 9:30 185

Formulation and environmental manipulation to enhance the larvicidal activity of entomopathogenic nematodes for control of insect pests of orchards

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Extended shelf life and rapid dispersion of entomopathogenic nematode (EPN) infective juveniles (IJs) in spray suspensions were the principal objectives of earlier EPN formulation development. Subsequently, formulation of EPNs for enhanced insecticidal control in greenhouses and field has been investigated by numerous researchers for control of several insect pests. However, there are few publications on the formulation of EPNs for improved control of orchard pests. Novel formulations that that slow desiccation of IJs are being developed that will facilitate increased use of EPNs as microbial control agents. For example, a sprayable gel substantially improved epn efficacy for control of the lesser peachtree borer, *Synanthedon pictipes*, on peach limbs. Control of overwintering cocooned codling moth, *Cydia pomonella*, larvae would drastically reduced damage caused by the moth following emergence in the spring. A wood flour foam formulation and formulated EPN infected-hosts, caused superior control of overwintering larvae on tree trunks and in mulch, respectively. These new formulations (e.g., sprayable gels or foams) may have widespread benefits for epn application, particularly for targeting pests that attack the tree aboveground. EPNs and other microbial control agents are ready made components of integrated management of orchard pest insects on a sustainable basis.

Keywords: Codling moth, lesser peach tree borer, entomopathogenic nematodes, formulation

Contributed Papers
Thursday, 8:00-10:15
Fahri Kuran

BACTERIA 4

Chair: Baltasar Escriche and Neil Crickmore

Contributed Paper Thursday, 8:00
186-STU

Novel members of the *repat* gene family and their expression in the midgut of *Spodoptera exigua* larvae in response to pathogens

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Previously, a new family of genes that showed overexpression after exposure to *Bacillus thuringiensis* Cry1Ca toxin and baculovirus infection was identified in the midgut of the Lepidoptera *Spodoptera exigua*. These 4 new genes were named *repat* base on their up-regulation in response to pathogen. Recently, 3 novel *repat* genes showed an increased expression in a *S. exigua* colony that had developed resistance to a *B. thuringiensis* formulate. An additional *repat* gene was described in yeast two hybrid experiments as REPAT1 interactor. In total, 8 members of the REPAT family were previously described.
In this study, transcriptome sequencing of *S. exigua* larvae using 454 sequencing revealed the expression of additional genes with homology to *repat* genes. Sequence analysis revealed the presence of at least 21 *repat* genes and 25 *repat-like* genes. RT-qPCR was applied in order to determine the expression profiles of these genes in the larval midgut under different conditions. *S. exigua* larvae were exposed to a single *B. thuringiensis* toxin (Cry1Ca), a *B. thuringiensis* formulate (Xentari™), *B. thuringiensist* cry- strain (that does not contain Cry toxins), *Enterococcus sp.* isolated from a *S. exigua* colony *(*gram positive), *Escherichia coli* (gram negative) and also conditions that affect the midgut physiology as Paraquat and a starvation condition. Expression information, as well as sequence information was combined to detect groups of genes that manifest similar expression patterns which could offer a potential insight into gene function and regulatory mechanisms.

Keywords: Bacillus, beet armyworm, bacteria, infection

Contributed Paper **Thursday, 8:15**
187-STU

Screening of *Bacillus thuringiensis* isolates against *Agrotis segetum* with specific focus on vegetative insecticidal proteins

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80 *B.t.* isolates were selected for a preliminary screening against *Agrotis segetum*. The 29 isolates from the Julius Kuhn-Institut (JKI), Germany, were isolated from insects and soils from Africa, Philippine and Germany. 48 isolates from the Centre of Biotechnology of Sfax (CBS), Tunisia, were isolated from soils. Stains *B.t. kurstaki* HD-1, *B.t. aizawa* HD-133 and *B.t. aizawa* from Xentari were used as reference.For testing the preliminary biological activity, the *B.t.* crystal/spore content was quantified and bioassays were conducted by feeding first instar larvae of *Agrotis segetum*. 56 isolates had more than 60% corrected mortality after three days. Six isolates caused a corrected mortality of at least 100%. The biochemical and molecular characterization of the most promising isolates was performed and will be discussed. In the next set of experiments the influence of vegetative insecticidal proteins (VIPs) on the larval development was investigated by adding the supernatant of the liquid medium harvested in the exponential growth phase to third instar larvae. The weight and the mortality were measured after 14 and 35 days. By Western Blot the presence of VIPs in the supernatant was proofed. Isolates, which produced VIPs showed a reduction in larval development and mortality. Additional histological investigations will be discussed. For further investigations a specific technique to quantify the VIP content was needed. Results on the development of monoclonal antibodies will be shown.
Keywords: Vegetative Insecticidal Proteins, Bacillus thuringiensis, Agrotis segetum

Contributed Paper **Thursday, 8:30 188**

Proteolytic processing of *Bacillus thuringiensis* Cry7Aa1 toxin and specific binding to brush border membrane vesicles of three sweetpotato weevil species (Coleoptera: Brentidae)

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Sweetpotato is a major staple for human consumption in some third world countries, and an important cattle feed in others. During dry season, crop losses can reach 100%, threatening food security, due to sweetpotato weevils, *Cylas spp*. (Coleoptera: Brentidae). *C. puncticollis* and *C. brunneus* are the most common species in East Africa, while *C. formicarious* is the most common species in North America and Asia. Current control strategies have not been effective against these pests justifying the need for new control methods. Recently, different Cry toxins have been found to be toxic to *Cylas spp*., with Cry7Aa1 being one of the most toxic. Little is known about the mode of action of Bt Cry toxins active against *Cylas spp.* in comparison to *B. thuringiensis* Cry1 toxins. Cry1 toxins are mostly toxic to lepidopterans, which exhibit a complex mode of action characterized by a proteolytic activation and specific receptor binding on the larval midgut. Our results demonstrate that the proteolytic activation of the Cry7Aa1 130 kDa protoxin with gut juice or brush border membrane vesicles (BBMVs) from weevil larvae rendered a 65 kDa protease-resistant polypeptide, similar to the *in vitro* activation with commercial bovine chymotrypsin or trypsin. The binding of the biotinylated Cry7Aa1 activated fragment was found to be specific to BBMV receptors in all three *Cylas spp.* This data indicates that the Cry7Aa1 toxin follows a similar mode of action process in weevil larvae as Cry1 toxins in lepidopteran larvae. Similar studies with other active proteins should be carried out to implement resistance management strategies.

Keywords: Sweetpotato weevil, *Bacillus thuringiensis*, Cry7Aa1, binding

Contributed Paper **Thursday, 8:45 189**

Analysis of the effect of mutations in domain II of *Bacillus thuringiensis* Cry3Aa toxin

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Bacillus thuringiensis Cry3 entomopathogenic toxins are specifically active against coleopteran pests. In this study toxicity of Cry3Aa and Cry3Ca toxins was assessed in *Leptinotarsa decemlineata* (Colorado potato beetle). The LC_{50} of Cry3Ca was four times lower (2.97 μg/ml) that the one of Cry3A (12.77 μg/ml) in a leaf-dip assay. We were interested in tackling the aspects of the mode of action of this toxin that accounts for the difference in toxicity between Cry3Aa and Cry3Ca, two toxins that share 74% identity of sequence. The role of proteolysis and pore formation was analyzed. Cry3Ca toxin was more readily proteolytically cleaved than Cry3Aa and it formed pores more efficiently. We have previously identified a stretch of amino acids in domain II of Cry3A toxin F_{342}HTRFQPGYYGNDSFN$_{357}$ which is able to prevent proteolytic processing and nearly abolishes pore formation. In this study we used site directed mutagenesis on specific residues of this stretch in Cry3A to make it identical to the one of Cry3Ca: F_{342}HSRLQPGYFGTDSFN$_{357}$. Results are discussed in terms of strategies to improve activity of Cry3 toxins

Keywords: *Bacillus thuringiensis*, Cry3A, mutagenesis, toxicity, Coleopteran

Contributed Paper **Thursday, 9:00 190**

Gene dosage analysis in the European Corn Borer *Ostrinia nubilalis* (Lepidoptera) using quantitative real-time PCR with SYBR Green detection

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The genome of the majority of pest species has been poorly studied. The determination of the number of copies of the resistance genes is of great importance to establish the proper genetic basis for a resistance pest management strategy. However, several factors, such as small size of the sample or tandem duplications, makes difficult the accurate determination of the amount of copies of a gene using traditional technologies (i.e. Southern blot or FISH analyses). The objective of the present work has been to set up the quantitative real-time PCR technique with SYBR Green detection to determine the number of gene copies of *cadherin*, which codifies for a *Bacillus thuringiensis* Cry toxin receptor, in *Ostrinia nubilalis* (Hubner) thoraxes from single individuals.
The capacity and robustness of the technique was first tested with genes located in the sexual chromosome X in the model organism *Drosophila melanogaster* (Diptera). The system was then validated in *O. nubilalis* (Lepidoptera) by detecting genes located in the sexual chromosome Z, and finally was applied to study the *cadherin* gene in this insect. The autosomal single copy gene *RpS3*, which codifies for a ribosomal protein, was used as a control gene. The technique allowed discriminating the occurrence of one, two or more copies of the studied genes per genome. The *cadherin* was detected as an autosomal single copy gene in *O. nubilalis*. The results pointed to the suitability of the technique for rapid and accurate estimates of the copy number of any gene in any insect species.

Keywords: Drosophila, cadherin, sex chromosome

Contributed Paper **Thursday, 09:00 191**

Restoration of the crystallisation of altered delta-endotoxins Cry1Ac, by the promotion of their *in vivo* integration into the *Bacillus thuringiensis* native crystal

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Cry1Ac is one of the most studied *Bacillus thuringiensis* delta-endotoxins. Structurally, the latter are divided into two domains: the N-terminal half corresponding to the toxic component and the C-treminal half corresponding to the region responsible for the crystal formation. We engineered Cry1Ac delta-endotoxins modified in their N-terminal part and studied the effect of such modifications on crystallisation and delta-endotoxin production. When expressed in an acrystalliferous *Bacillus thuringiensis* strain, Cry1Ac* and Cry1AcD, a 4 point-mutation and a deleted Cry1Ac delta-endotoxins respectively, could not form crystals. However, when expressed in a crystalliferous strain, these altered proteins were shown to interact with the endogenous delta-endotoxins and co-crystallise with them forming atypical crystals observed by electron microscopy. This co-crystallisation of the altered delta-endotoxins with the endogenous ones led to a decrease in delta-endotoxin production (28 %) by the corresponding recombinant *Bacillus thuringiensis* strains. This ability of altered delta-endotoxins containing intact C-terminal part to co-crystallise with native ones could be exploited to promote the crystallisation of foreign proteins by fusing them with C-terminal part of Cry1A delta-endotoxins.

Keywords: Bacillus thuringiensis, delta-endotoxins, cry gene, C-terminal portion, co-crystallisation

Contributed Paper **Thursday, 9:30 192-STU**

Role of Cry3Aa domain II loop 1 in the mode of action of Cry3Aa toxin

Fernando Zúñiga[1]; **Isabel Gómez**[1]; **Ernesto Ortiz**[1]; **Alejandra Bravo**[1]; **Mario Soberón**[1]
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Cry toxins of *Bt* are pore-forming toxins, their primary action is to lyse midgut epithelial cells in target insect. The mode of action of Cry toxins has been studied principally in lepidopteran insects where they undergo a complex sequential binding mechanism with at least two different receptor molecules. The first binding event with a cadherin insect molecule facilitates the formation of a pre-pore oligomeric structure that binds then to GPI-anchored receptors. Domain II loop regions have been recognized as key aminoacid regions involved in receptor interaction. In the case of Cry3Aa, mutagenesis of domain II loop regions showed that loop 1 and loop 3 are involved in binding and toxicity. To determine the role of loop 1 region in the oligomerization and toxicity of Cry3Aa, we isolated, using a M13 phage display Camel Fv library, Fv molecules that bind Cry3Aa loop 1. In this presentation we will present the partial characterization of these Fv molecules in Cry3Aa oligomerization assays and as binding competitors to *Tenebrio molitor* brush border membrane vesicles.

Keywords: Cry3A toxin, phage display, camel antibodies

Contributed Paper **Thursday, 9:45 193**

Identification of key regulators controlling expression of the Fit insect toxin in the root-associated biocontrol pseudomonad CHA0

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Pseudomonas fluorescens CHA0 is a root-associated biocontrol agent of major soil-borne diseases of crop plants caused by fungal and oomycete pathogens. Remarkably, the plant-beneficial pseudomonad is also endowed with potent systemic and oral insecticidal activity that depends on the production of a large protein toxin termed Fit (for *P. fluorescens* insecticidal

toxin). The Fit toxin gene *fitD* is flanked upstream by the *fitABC* genes and downstream by the *fitE* gene that encode the ABC transporter, transmembrane fusion and outer membrane efflux components of a type I protein secretion system predicted to function in toxin export. The *fitF*, *fitG*, and *fitH* genes located downstream of *fitE* encode regulatory proteins having domain structures typical of signal transduction histidine kinases, LysR-type transcriptional regulators, and response regulators, respectively. In this work, the role of FitG and FitH in the regulation of the expression of the fit locus genes was investigated using q-RT-PCR and GFP-based reporter fusions to the promoter regions of the fit genes. Our findings indicate that FitH represses the expression of the toxin gene *fitD* in a FitG-dependent manner. Interestingly, although transcribed independently of *fitD*, the *fitABC* operon and the *fitE* gene are also regulated in a FitH and FitG-dependent manner. FitH-dependent repression of *fitD* could also be confirmed at the translational level. The overexpression of the Fit toxin in the *fitH* mutant was correlated with a higher toxicity in Galleria larvae. These findings therefore strengthen the idea that fit genes flanking fitD are important for the FitD-dependent insecticidal activity of CHA0.

Keywords: Pseudomonad CHA0 Fit insecticidal toxin

Contributed Paper **Thursday, 10:00** **194**

Biochemical basis of field-isolated resistance to *Bacillus thuringiensis* Cry2A insecticidal proteins in *Helicoverpa* species

Silvia Caccia[1], Carmen Sara Hernández-Rodríguez[1], Rod J. Mahon[2], Sharon Downes[3], William James[2], Nadine Bautsoens[4], Jeroen Van Rie[4] and Juan Ferré[1]

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In Australia, a transgenic variety of cotton expressing Cry1Ac and Cry2Ab (Bollgard II) comprises at least 80% of the total cotton area. Prior to the widespread adoption of Bollgard II, the frequency of alleles conferring resistance to Cry2Ab in field populations of *Helicoverpa armigera* and *Helicoverpa punctigera* was significantly higher than anticipated. Colonies established from survivors of F_2 screens against Cry2Ab are highly resistant to this toxin, but susceptible to Cry1Ac. Bioassays performed with surface-treated artificial diet on neonates of *H. armigera* and *H. punctigera* showed that Cry2Ab resistant insects were cross-resistant to Cry2Ae while susceptible to Cry1Ab. Binding analyses with ^{125}I-labeled Cry2Ab were performed with brush border membrane vesicles from midguts of Cry2Ab susceptible and resistant insects. The results of the binding analyses correlated with bioassay data and demonstrated that resistant insects exhibited greatly reduced binding of Cry2Ab toxin to midgut receptors, whereas no change in ^{125}I-labeled-Cry1Ac binding was detected. As previously demonstrated for *H. armigera*, Cry2Ab binding sites in *H. punctigera* were shown to be shared by Cry2Ae, which explains why an alteration of the shared binding site would lead to cross-resistance between the two Cry2A toxins. This is the first time that a mechanism of resistance to the Cry2 class of insecticidal proteins has been reported. Because we found the same mechanism of resistance in multiple strains representing several field populations, we conclude that binding site alteration is the most likely means that field populations evolve resistance to Cry2 proteins in *Helicoverpa* spp.

Contributed papers
Thursday, 8:00-10:00
Nihat Turan 2

VIRUSES 5

Chairs: Eric Haas Stapleton and MartinErlandson

Contributed Paper **Thursday, 08:00** **195**

***Mamestra configurata* nucleopolyhedrovirus strain variation at the genome sequence level**

Martin Erlandson[1]; Matthew Links[1]; Ajaykummar Maghodia[1]; Cam Donly[1]; Dwayne Hegedus[1]; David Theilmann[1]

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Previously we sequenced the complete genomes of three *Mamestra configurata* nucleopolyhedrovirus (MacoNPV) geographic isolates and found that they constitute two different virus species, MacoNPV-A and –B. The two MacoNPV-A isolates 90/2 and 90/4 had a high degree of identity, but also had several gene deletions/insertions and were significantly different with respect to virulence. In addition to the sequenced genomes we had a substantial number of MacoNPV isolates from bertha armyworm (BAW) populations collected over a wide geographic area of western Canada from several temporal outbreak cycles of this noctuid pest. This collection offered a unique opportunity to further determine the level of genetic variation within these virus populations. MacoNPV isolates were derived from single-infected-cadavers from field-collected larvae and genomic DNA was purified and subjected to 454 pyrosequencing (Roche-Genome Sequencer FLX System), and the DNA sequence assembled using Nuebler software. We generated the complete genome sequence for an additional ten MacoNPV-A isolates with genomes ranging in size from 154,408 to 164,221 bp with 20 to 40 fold coverage. The genes with the highest degree of variability among the isolates included the MacoNPV-A *bro* genes –b, -c and –f, and their flanking ORFs. The MacoNPV-A *bro-a* gene was absent in one of the isolates, MacoNPV-S97/AH3d, as it was in MacoNPV-A (90/4). MacoNPV-A isolate 94/2 had an insertion of five ORFs with homology to the same Xec-nGV gene homologues that were found in MacoNPV-B, but this insertion occurred in a different region of the genome than was observed for MacoNPV-B. The virulence of these isolates was also compared in a time-to-mortality bioassay in BAW larvae to determine their possible phenotypic differences.

Keywords: Baculovirus, genomics

Contributed Paper **Thursday, 8:15**
196-STU

Comparative genomics of four isolates of *Cydia pomonella* Granulovirus (CpGV)

Karolin E. Eberle[1]; **Pit Radtke**[2]; **Johannes A. Jehle**[1]
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The *Cydia pomonella* Granulovirus (CpGV) isolate CpGV-M1 was one of the first fully sequenced granulovirus genomes. Further CpGV isolates from different geographic regions had been previously identified by restriction analysis. By phylogenetic analysis based on the *polh/gran* and *lef-8* genes, CpGV isolates could be recently grouped into genome types A to E, replacing the previous classification based on the geographic origin. Four CpGV genome types were completely sequenced, CpGV-I12 (type D genome), -S (type E genome), -I07 (type C genome) and compared to CpGV-M (type A genome), which was re-sequenced as reference. Genome analysis revealed differences in genome size and genetic content between the four isolates. Several insertions and deletions ranging from few nucleotides to 2.5 kbp were found. Regarding the site of these indel mutations, it is striking that the genome regions between 18-22 kbp and 50-60 kbp reveal a multiplicity of insertions, deletions and duplication events when comparing the four genomes, suggesting that these events are associated with repeated sequence motifs. Analysis of these genomic rearrangements, ORF content and codon usage give insight in the genomic variety and plasticity of CpGV genomes as well as into the evolutionary forces driving the micro-evolution of baculovirus genomes.

Keywords: Baculovirus, CpGV, whole genome sequencing

Contributed Paper **Thursday, 8:30**
197-STU

Comparative effects of "defective" genotypes in NPV populations: their roles in SfMNPV vs. SeMNPV

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The *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) isolate from Florida, US (SeUS2) is a complex mixture of genotypes. The genotypic structure of this isolate is very similar to that of the *S. frugiperda* MNPV isolated from Nicaragua (SfNIC), which is composed of at least nine genotypes. Both isolates present defective genotypes with similar deletions that lack peroral infectivity. The SfNIC defective genotype (SfNIC-C) plays a functional role in the transmissibility of the viral population since its presence increased the pathogenicity of OBs in co-occluded mixtures of complete and defective genotypes. The aim of this study was to determine the functional role of the defective genotype SeUS2-C, that is not infective per os, in the SeUS2 isolate. For this, different experimental populations were constructed that included the complete genotype SeUS2-A and the deletion mutant genotype SeUS2-C at different proportions in OB mixtures or co-occluded genotypic mixtures. The SeUS2-C was the most abundant isolated genotype in cell culture accounting for ~75% of plaques. However, the proportion of SeUS2-C in the viral population as determined by semiquantitative PCR was ~25%. SeUS2-A OBs were as pathogenic as wild-type OBs. As predicted, the potency of the mixtures of SeUS2-A OBs and SeUS2-C OBs was dependent on the proportion of SeUS2-A present in the mixture. In contrast, the co-occluded mixtures that included the defective genotype SeUS2-C at the proportions of 10 and 25%, respectively, were as pathogenic asSeUS2-A. However, the presence of a higher proportion of SeUS2-C in the mixtures reduced the biological activity of OBs. We conclude that the defective genotype SeUS2-C appears to persist as a parasitic genotype in the SeUS2 population. This contrasts with the role of defective genotypes in the SfMNPV population that potentiate OB potency.

Keywords: Defective genotyes, SfMNPV, SeMNPV, transmissibility

Contributed Paper **Thursday, 8:45** **198**

Molecular genetics of the densovirus resistance genes, *nsd-1* and *nsd-2*, in *Bombyx* silkworms

Keiko Kadono-Okuda[1]; **Dorington Okeyo Ogoyi**[1]; **Kurako Kidokoro**[1]; **Masahiro Ajimura**[1]; **Eiichi Kosegawa**[1]; **Yutaka Banno**[2]; **Hideaki Maekawa**[3]; **Yumiko Nakajima**[3]; **Keiji Yukuhiro**[1]; **Katsuhiko Ito**[1]; **Kimiko Yamamoto**[1]; **Kazuei Mita**[1]
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Bombyx mori densovirus (BmDNV) belongs to Parvoviridae and multiplies in the columnar cell nuclei of the *Bombyx* midgut. It is classified into two species, BmDNV-1 and BmDNV-2/-Z (Parvo-like virus). Some silkworm strains are absolutely resistant against BmDNV-1 and/or BmDNV-2. Three unlinked mutations, *nsd-1*, *Nid-1* and *nsd-2*, which were discovered in different *Bombyx* strains, control nonsusceptibility against the infection by BmDNV-1 or 2. We undertook map-based cloning of the genes, *nsd-1*, *Nid-1* and *nsd-2*.
So far we have isolated *nsd-1* and *nsd-2*. The *nsd-1* was predicted to encode a one-pass transmembrane protein expressed only in the midgut, and an amino acid substitution was observed in resistant strains. The virus-resistance caused by *nsd-2* is due to a 6 kb deletion where the gene is located. The gene encodes a 12-pass transmembrane protein expressed only in the midgut columnar cells. Germline transformations with wild type transgenes, $+^{nsd-1}$ and $+^{nsd-2}$, expressed in the midgut restored susceptibility, showing that the mutated membrane proteins are responsible for

resistances. We identified the *nsd-1* and *nsd-2*-homologous genes in *B. mandarina* which is a probable wild species of *B. mori*. Their genotypes of resistance or susceptibility to the densoviruses coincided with the responses to the viral inoculation. *B. mandarina* collected from various places in east Asia and many local varieties of *B. mori* were genotyped for *nsd-1* and *nsd-2*.
We will discuss the relationship among the viruses, *B mori* and *B. mandarina* from the results of genotyping of these resistance genes.

Keywords: Densovirus registant, *Bombyx*

Contributed Paper Thursday, 9:00 199

Dynamics of the salivary gland hypertrophy virus in laboratory colonies of *Glossina pallidipes* (Diptera: Glossinidae)

Abd-Alla Adly[1]; **Kariithi Henry**[1]; **Parker Andrew**[1]; **Robinson Alan**[1]; **Kiflom Musie**[2]; **Bergoin Max**[3]; **Vreysen Marc**[1]
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Many species of tsetse flies are infected by a virus that causes salivary gland hypertrophy (SGH) and the virus isolated from *Glossina pallidipes* (GpSGHV) has recently been sequenced. Flies with SGH have a reduced fecundity and fertility. To better understand the impact of this virus in a laboratory colony of *G. pallidipes*, where the majority of flies are infected but asymptomatic, and to follow the development of SGH in the offspring of symptomatic infected flies, we examined the progeny of tsetse flies reared under different conditions. The results show that stress in the form of high fly density in holding cages (180 flies/cage) and high temperature (30°C) in the holding room did not affect the prevalence of the SGH. The virus is excreted in the saliva and there is a strong correlation between the infection status (negative, slight or strong by PCR) and the numbers of virus particles released into the blood on which the flies were fed. On average, around 10^2 and 10^7 virus particles were found in the blood after feeding asymptomatic or symptomatic infected flies respectively. The results also show that the progeny of asymptomatic parents did not develop SGH when fed on clean blood, while the progeny of symptomatic female flies mated with asymptomatic males developed a high rate of SGH (65% in male and 100% in females) and these flies were sterile. Feeding the flies on new blood at every feed for three generations caused a significant reduction in the virus copy number in these flies when compared with the virus copy number in flies fed under the normal feeding regime. The results of these studies allowed the initiation of colony management protocols that aim to minimize the risk of horizontal transmission and to enable the establishment of colonies with a low virus prevalence or possibly even those that are virus free.

Keywords: Tsetse, Salivary Gland Hypertrophy virus (SGHV), Transmission, Sress factor, Virus Management

Contributed Paper Thursday, 9:15 200

Do persistent NPV infections in *Spodoptera exigua* affect host fitness?

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Vertical transmission of covert NPV infections is a common phenomenon in field populations of the beet armyworm (*Spodoptera exigua*, Huber) in the horticultural region of Almería (Spain). The genotypic diversity of the *Spodoptera exigua* nucleopolyhedrovirus population in this region has proved to be extremely high. A recent study suggests that some of these genotypic variants are involved in vertically-transmitted infections whereas other genotypes are involed in horizontally-transmitted infections. These findings led us to examine whether host-pathogen interactions are associated with a range of fitness correlates or tradeoffs in either the insect or the virus. First, we demonstrated that *S. exigua* adults that survived a virus challenge (9×10^3 OB/ml) presented a persistent sublethal infection detected by RT-PCR (85 - 100% of insects were positive for viral transcripts of the *DNA-polymerase* gene) following inoculation by vertically transmitted genotypes. A vertically transmitted genotype was then selected to examine the effects of larval age at inoculation and the dose of virus ingested on the prevalence of persistent virus infections in adults. Larvae of four different larval instars (L_2, L_3, L_4, and L_5) were dosed with four different concentrations that resulted in 20 to 80% of NPV-mortality. A similar trend was observed for each larval stage, the higher the dose of inoculum, the higher the proportion of adults that proved positive for viral transcripts. Finally, we observed that persistent infections reduce the fitness of individuals which survive the infection in terms of host developmental parameters (larval developmental time, pupal weight, adult fecundity, fertility, and longevity) in an insect colony in which a persistent infection had been induced compared to mocked-infected insects. On-going experiments are being performed to examine the biological costs of harbouring covert infections.

Keywords: Vertical transmission, Spodoptera exigua, SeMNPV, fitness, RT-PCR, larval stage, developmental parameters

Contributed Paper Thursday, 9:30 201

Variation in life history and flight morphological traits in Speckled Wood (*Pararge aegeria*) butterflies infected with a baculovirus

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Sub-lethal impacts are known to affect the insect-host relationship and have an important role in describing host dynamics. The impact of sub-lethal infections of pathogens on life history traits of affected hosts has been understudied in natural or semi-natural systems. The Speckled Wood (*Pararge aegeria*) is a satyrine butterfly that is common in temperate zones and has been extensively used as a model system for evolutionary ecology studies. It is known that the deployment of the immune system within this species, as with other invertebrates, is energetically costly and may result in trade-offs with fitness-related traits. In this study, we investigated the sub-lethal effect of exposure to *Autographa californica* multiple nucleopolyhedrovirus (*Ac*MNPV) on life history and flight morphological traits of *P. aegeria*.
Larvae were inoculated with increasing doses of *Ac*MNPV and measurements made of life history and flight morphology traits. Generally, larvae exposed to virus took longer to develop to pupae and larval mass acquisition per day was significantly reduced in viral exposed larvae. However, viral exposed larvae were able to attain the same pupal mass and their duration as pupae was the same as controls. Forewing length, forewing aspect ratio, dry thorax mass and forewing loading were related to sex and bioassay differences but there was no evidence of any viral impact on these measures. Adult male butterflies had significantly less basal wing melanisation when exposed to virus compared to control males but there was no difference between females. Implications for population dynamics of *P. aegeria* are discussed.

Keywords: Host-pathogen interactions, baculovirus, speckled wood, sub-lethal, life-history traits

Contributed Paper **Thursday, 9:45** **202**

Transmission strategy is correlated with pathogenicity and disparate virulence and productivity traits in an insect nucleopolyhedrovirus

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The prevalence of sublethal infections of *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) was quantified in natural populations of *S. exigua* in Almeria, Spain, during 2006 and 2007. Of 1045 adults collected, 16% proved positive for viral *polyhedrin* gene transcripts by RT-PCR. The prevalence of covert infection varied significantly according to sex and sample date. Of 1660 progeny of field-collected insects, lethal disease was observed in 10-33% of offspring of transcript-positive females and 9-49% of offspring of transcript-negative females. Isolates associated with vertically-transmitted infections were characterized by restriction endonuclease analysis using *Bgl*II and compared with isolates believed to be horizontally-transmitted. Insects from a sublethally-infected Almerian colony were ~3.4-fold more susceptible to infection than healthy insects from a Swiss colony. Horizontally-transmitted isolates were significantly more pathogenic than vertically-transmitted isolates in insects from both colonies. Mean speed of kill varied between isolates by >20 h, whereas mean occlusion body (OB) production varied by 3.8-fold among isolates. Intriguingly, all three horizontally transmitted isolates were very similar in speed of kill and OB production, whereas all three vertically transmitted isolates differed significantly from one another in both variables, and also differed significantly from the group of horizontally-transmitted isolates in one or both variables. We conclude that key pathogenicity and virulence traits of SeMNPV isolates vary according to their principal transmission strategy.

Keywords: Sublethal infections, Vertical transmission, horizontal transmission, SeMNPV, *Spodoptera exigua*, pathogenicity, virulence, productivity

Thursday, 8:00-10:00
VIEW POSTERS
Fungi (Authors stand by posters when not in session)

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

Thursday, 10:30-12:30
Hasan Turan
SOCIETY for INVERTEBRATE PATHOLOGY
Annual Business Meeting
Presentation by Elizabeth W. Davidson: Why do we keep coming to the SIP meeting? Delivered by James Harper.

12:30-14:00 **LUNCH at KTU SAHIL**

13:00-14:00
Demonstration Room
Business Meeting
Student and Post Docs

Thursday , 14:00-16:00
VIEW POSTERS
Virus, Bacteria and Fungi
(Authors stand by posters)

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

Contributed Papers
Thursday, 16:30-17:45
Fahri Kuran
VIRUSES 6

Chairs: Karyn Johnson and Kelli Hoover

Contributed Paper Thursday, 16:30 203

Anti-viral defenses contribute to developmental resistance to LdMNPV in gypsy moth

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Developmental resistance to LdMNPV within an instar is well documented in the gypsy moth, *Lymantria dispar*. The LD_{50} in newly-molted fourth instars is 18-fold lower than in the middle of the instar (48-72 h post-molt). Using a recombinant of LdMNPV expressing *LacZ*, we examined the key steps of pathogenesis in the host to explore mechanisms of developmental resistance. At the midgut level, we observed reduced primary midgut infections in mid-fourth instars indicating increased sloughing of infected cells. Additional barriers were observed as the virus escaped the midgut. Mid-fourth instars had relatively higher numbers of melanized foci of infection associated with the midgut, apoptotic tracheal epidermal cells and hemocytes, and reduced numbers of infected hemocytes later in infection. Our results show that the co-evolutionary relationship between *L. dispar* and LdMNPV has resulted in both midgut-based and systemic anti-viral defenses that are age-dependent. This age related susceptibility may contribute to maintenance of LdMNPV in nature and could influence efficacy of the virus as a microbial control agent.

Keywords: Baculovirus, resistance, *Lymantria dispar*

Contributed Paper Thursday, 16:45 204

Hemocytes proliferate in response to inactivated baculovirus

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We investigated the proliferative response of hemocytes to baculovirus infection. Systemic infection of permissive *Trichoplusia ni* larvae with *Autographa californica* M Nucleopolyhedrovirus (*Ac*MNPV) increased the numbers of circulating hemocytes relative to vehicle-inoculated larvae. To evaluate the impact of *Ac*MNPV on hemocyte proliferation in the absence of bystander effects associated with an active infection, we quantified the proliferation of carboxyfluorescein succinimidyl ester (CFSE)-stained *T. ni* hemocytes exposed to heat-inactivated *Ac*MNPV budded virus (iBV) or vehicle. CFSE is a fluorescent dye that stains the cytosol and is partitioned equally with each cell division. Consequently, the fluorescence intensity of CFSE is diminished by half each time a cell divides. There was a significant reduction in CFSE mean fluorescence intensity of CFSE-stained hemocytes cultured for three days in the presence of iBV, relative to vehicle, when assayed using fluorescence microscopy. Using flow cytometry, 48.6% of the iBV-exposed hemocytes divided *in vitro* more than twice, compared to 16.2% for vehicle-exposed hemocytes. To quantify hemocyte proliferation kinetics *in vivo*, we developed a method for transferring hemocytes stained *ex vivo* with CFSE to recipient *T. ni* larvae. One-fifth of the transferred CFSE-stained hemocytes were recovered from larvae after circulating for 48 h *in vivo*. Co-transfer of iBV with CFSE-stained hemocytes resulted in a 3-fold reduction in the number of CFSE-positive hemocytes. The results suggest that iBV stimulated hemocyte proliferation both *in vitro* and *in vivo*. These studies provide a platform for quantifying proliferative responses to baculovirus infection in permissive and resistant hosts.

Keywords: Baculovirus hemocyte proliferation

Contributed Paper Thursday, 17:00 205

Baculovirus infections alter the titers of detoxification enzymes in *Spodoptera littoralis* Boisd. (Lepidoptera: Noctuidae)

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Two detoxification enzyme groups (Carboxylesesterases and Glutathion-S-transferase) were studied in the egyptian cottonworm (*S. littoralis* Boisd.), infected with SpliNPV-TR1. Neonate larvae and third instar larvae were infected with doses of 3.000 OBs and 250.000 OBs of SpliNPV-TR1. The bioassays were conducted using a droplet feeding method. After infection, larvae were collected once every twenty four hours until first larvae death occured. The carboxylesterase activity and Glutathion-S-transferase activity was determined spectrophotometrically using the substrates, α-naphyl acetate, α-naphyl butrate and CDNB, DCNB respectively. The results showed that detoxification enzyme concentrations were significantly higher in SpliNPV-TR1 infected larvae than uninfected larvae.

Keywords: *Spodoptera littoralis*, baculovirus, detoxification, carboxylesesterases, glutathion-S-transferase

Contributed Paper Thursday, 17:15 206

Effects of silencing apoptosis regulatory genes on Sindbis virus replication and dissemination in *Aedes aegypti*

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Sindbis virus (SINV) is a member of the *Togaviridae* family, and is the type species of the genus *Alphavirus*. SINV is associated with occasional outbreaks of disease in Africa, Europe and Asia. SINV is an arthropod-borne virus (arbovirus) which is normally vectored by mosquito species in the genus *Culex*, but is also capable of being vectored by the yellow fever mosquito, *Aedes aegypti*. Since *Ae. aegypti* is the major vector of yellow fever and dengue fever, and since SINV has been well characterized at the molecular level (including the development of gene expression systems based on the SINV genome), SINV infection of *Ae. aegypti* is often used as a model to study arbovirus-mosquito interactions. We are

interested in determining whether apoptosis plays a role in the ability of arboviruses to replicate in mosquitoes. Genes which either positively or negatively regulate apoptosis in *Ae. aegypti* were silenced by RNA interference in adult female mosquitoes, and the mosquitoes were then fed a blood meal containing the SINV infectious clone p5'dsMRE16ic expressing green fluorescent protein (MRE/GFP). Effects were observed on the both the occurrence and intensity of expression of GFP in various mosquito tissues, depending on the gene silenced. Increased caspase activity and mosquito mortality were also observed following silencing of a negative regulator of caspase activity, even in the absence of virus infection. These results suggest that apoptosis may influence SINV replication in *Ae. aegypti*.

Keywords: Mosquito, alphavirus, arbovirus, apoptosis

Contributed Paper **Thursday, 17:30 207**

Prospects for Managing Turfgrass Insect Pests with Baculoviruses

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Microbial insecticides presently constitute < 0.1% of the $500 million U.S. turfgrass insecticide market but potentially could play a much greater role for suppressing insect pests of lawns, golf courses, and sport fields. We evaluated effectiveness of *Agrotis ipsilon* multiple nucleopolyhedrovirus (AgipMNPV), with or without adjuvants, for short-term and season-long control of the black cutworm, *Agrotis ipsilon*, in golf course habitats, as well as potential interactions with alkaloids present in relatively insect-resistant endophytic grasses. Our results showed AgipMNPV to have good potential as a knockdown or short- term preventive bio-insecticide targeting early instars, but also identified limitations to it providing extended control in this closely-mowed and irrigated perennial system. The virus is not deactivated when ingested on endophytic grasses, but reduced feeding on or avoidance of such grass may affect likelihood of larvae consuming a lethal dose. Prospects and hurdles to managing other turfgrass pests with biological insecticides will be discussed.

Keywords: AgipMNPV, Baculoviruses, Turfgrass pests

Contributed Papers
Thursday, 16:30-18:15
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BACTERIA 5

Chairs: Hyun-Woo Park and Gregory T. Sullivan

Contributed Paper **Thursday, 16:30 208**

Genetic assessment of the virulence factors of *Serratia proteamaculans* strains that cause atypical disease symptoms in the grass grub *Costelytra zealandica*

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Some strains of *Serratia entomophila* and *S. proteamaculans* cause amber disease of the New Zealand grass grub *Costelytra zealandica* (Coleoptera: Scarabaeidae). The disease determinants are encoded on a large 153,404-bp plasmid, termed pADAP for amber disease associated plasmid. Occasionally field isolated *C. zealandica* larvae of an unusual colouration are observed. Bacteria were isolated from these larvae and assessed for ability to induce disease in grass grub. Eight strains causing unusual disease phenology were isolated. 16sRNA DNA sequence analysis identified all strains as *S. proteamaculans*. In bioassays, 6 strains caused high mortality of grass grub within a 7 day period. Although these strains caused grubs to die more quickly than *S. entomophila*, only 80-90 % of larvae died, while the remaining larvae did not become diseased. Two further strains were identified that exhibited high mortality within a seven day duration. To identify potentially novel virulence genes, virulent strains were then assessed for plasmid content and pADAP-encoded genes, including components of the pADAP encoded Toxin complex *sep* virulence associated region and the antifeeding prophage. All strains were found to contain variants of pADAP. Two different Toxin complex gene clusters were identified, one of which was found to be highly virulent to *C. zealandica* larva. Of interest strains that contained the more potent Toxin complex gene cluster contained no antifeeding prophage. The diversity and possible ecological significance of pADAP plasmid variants are discussed.

Keywords: Serratia, *sep*, *afp*, pADAP

Contributed Paper **Thursday, 16:45 209**

Bacteria isolated from overwintering *Hyphantria cunea* (Lepidoptera: Arctiidae) pupae as potential entomopathogens

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Hyphantria cunea larvae feed on a wide range of forest and fruit trees. More than 250 species have been recorded as host plants. Overwintering pupae of *H. cunea* were collected from hazelnut plantations in Samsun province, Turkey in 2008 and 2009 to identify potential biological control agents. Bacteria isolated from pupae were identified by the use of the computer-assisted microbial identification system (MIS). The seven bacteria isolated were *Pseudomonas*

fluorescens biotype C, *P. agarici*, *P. putida* biotype B, *P. savastanoi fraxinus*, *Bacillus-cereus*-GC subgroup B, *Enterobacter intermedius and Enterecoccus mundtii*. A preliminary study determined that *Pseudomonas fluorescens* biotype C had the highest efficacy as an entomopathogen of *H. cunea* pupae.

Keywords: Hazelnut, *Hyphantria cunea*, Biological control, Entomopathogenic bacteria, *Pseudomonas* spp.

Contributed Paper Thursday, 17:00 210

Diversity and transmission of *Wolbachia* in spider mites

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Wolbachia is a bacterium infecting two-thirds of arthropod species. It is well known for manipulating host reproduction, but also beneficial and detrimental effects on host fitness are known, and the application of *Wolbachia* as a bio-control tool is currently investigated. Generally, it is assumed that *Wolbachia* is transmitted vertically, however, evidence is accumulating that horizontal transmission occurs as well. We present a study investigating *Wolbachia* diversity and transmission in spider mites (Acari: Tetranychidae). Molecular data reveal a high rate of recombination among *Wolbachia* strains. Using *Wolbachia* strains isolated from 64 individual mites we show that recombination plays a significantly higher role in generating diversity than point mutations, as recombinational events are 7.5 to11 times more likely to occur than point mutations. This finding is discussed in comparison to recombination rates found in other bacteria and in relation to the mode of transmission of *Wolbachia*.

Keywords: Wolbachia, spider mites, host-parasite

Contributed Paper Thursday, 17:15 211

Identification of a biosynthesis gene cluster involved in *Pseudomonas entomophila* virulence toward *Drosophila*

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Pseudomonas entomophila is a recently identified bacterium that is able to infect and kill *Drosophila*. *Drosophila* has evolved highly efficient innate defense mechanisms to combat microbial infection, making *P. entomophila* a good model to decipher pathogen strategies to subvert host defenses. Analysis of the *P. entomophila* genome identified a number of highly diverse genes that could be potential virulence factors and toxic secondary metabolites. Previous studies have determined the central role of the two-component system GacS/GacA, which controls among other proteins the production of the metalloprotease AprA that is associated with *P. entomophila* resistance to antimicrobial peptides. Here we report the isolation of an eight-gene operon, identified in a genome-wide mutagenesis screen, involved in *P. entomophila* virulence toward *Drosophila*. Transposon insertion into this cluster dramatically reduced gut cytopathologies and lethality of *Drosophila*, without affecting bacterial growth. The mutant induces the *Drosophila* gut immune response to a level similar to wild-type bacteria, suggesting that only the cytotoxicity of the mutant is impaired. The affected operon consists of genes encoding for enzyme often found in LPS biosynthesis cluster. Preliminary analysis comparing the LPS of the wild-type strain and mutant strain support this hypothesis. A particular modification of the LPS may represent an additional facet of *P. entomophila* virulence.

Keywords: Pseudomonas, Drosophila, Virulence

Contributed Paper Thursday, 17:30 212

The effects of entomopathogenic bacteria on *Thaumetopoea pityocampa* (Lepidoptera: Thaumetopoeidae)

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Thaumetopoea pityocampa is an important pest species of pine woods in central and southern Europe, including Turkey. This study investigated *Bacillus megaterium*, *Pseudomonas fluorescens* biotype C and *P. putida* biotype B as potential biological control agents of *T. pityocampa*. The bacteria used were from stock culture and were identified by the computer-assisted microbial identification system (MIS). The experiment was carried out in a completely randomized plot design under laboratory conditions. Larvae, leaves and nests were sprayed or dipped with the three bacterial suspensions (10^8-10^9 cfu/ml). There were highly significant differences between the bacterial applications and the control for the mortality of larvae. After the bacterial suspensions were applied to the larvae, there was a slowing down of movements, cessation of feeding and then brown-black discoloration of the diseased larvae, followed by death.

Keywords: Pine processionary caterpillar, Biological control, *Bacillus megaterium*, *Pseudomonas putida*, *Pseudomonas flourescens*

Contributed Paper Thursday, 17:45 213

Evidence of the involvement of 358E, 498A and 571C in the toxicity and binding of a new Cry1Ac-

type protein of *Bacillus* thuringiensis to E. kuehniella receptors

Imen saadaoui[1]; **Nabil Miled**[2]
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Bacillus thuringiensis is a gram-positive bacterium which produces, -endotoxins.δduring sporulation, crystalline inclusions containing one or more The latter are selectively toxic against a wide variety of insects, including important pests [1]. BLB1 is a Tunisian *B. thuringiensis* strain highly toxic against lepidopteron larvae of *E. kuehniella* compared to HD1 strain [2 and 3]. The investigation of its *cry* genes showed the presence of a new type of *cry1Ac* gene harboring three changes compared with that of HD1. The bioassay of their correspondent proteins showed that BLB1 Cry1Ac is at least two times more toxic than that of HD1. Furthermore, the study of the structural effect of these mutations suggested that they may stabilize key regions involved in the binding of the domain II and III to insect receptors [4]. To verify this hypothesis, we investigated the different steps of the mode of action of Cry toxin. Indeed, we compared the proteolysis, the "*in vitro*" and «*in vivo*» analysis of the binging characteristics between the two Cry1Ac toxins to determine the effect of 358E, 498A and 571C in the mode of action of Cry1Ac protein. The comparative analysis of the proteolysis activation of the two toxins showed the same rate and stability of the two toxins. However, in situ binding, histopathological effect analysis at different times allowed concluding that the BLB1 Cry1Ac bind faster and with a higher affinity to the *E. kuehniella* receptors. These findings could explain the difference of toxicity between BLB1 and HD1 strains.

Keywords: *Bacillus thuringiensis, E. kuehniella*

18:15 **Buses leave for hotels**

18:30 **Buses return from hotels to KTU SAHIL**

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

20:00-00:30
BANQUET and AWARDS CEREMONY

We hope to see you in Halifax, Canada for SIP 2010!

AUTHOR INDEX
SIP 2010

22:30-00:30 Buses return to hotels**AUTHOR INDEX**

Abstract no: 136 indicates oral presentation; B-16 indicates poster presentation

Author	**Presentation/Poster**

A

Abstract no: 136 indicates oral presentation; B-16 indicates poster presentation

NOTES