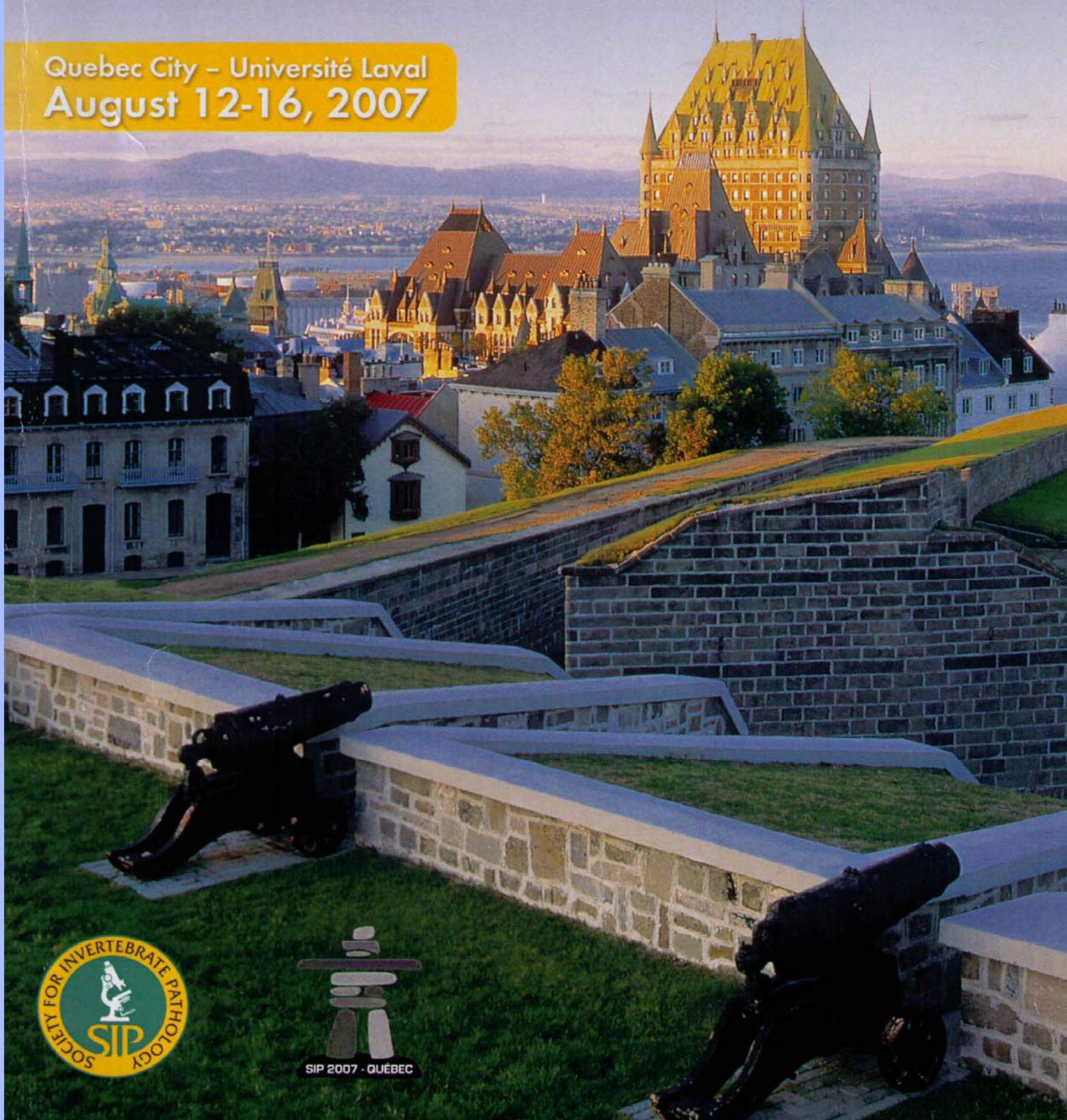
40TH ANNUAL MEETING
OF THE SOCIETY FOR INVERTEBRATE PATHOLOGY

AND

1ST INTERNATIONAL FORUM
ON ENTOMOPATHOGENIC NEMATODES AND SYMBIOTIC BACTERIA

Quebec City – Université Laval
August 12-16, 2007



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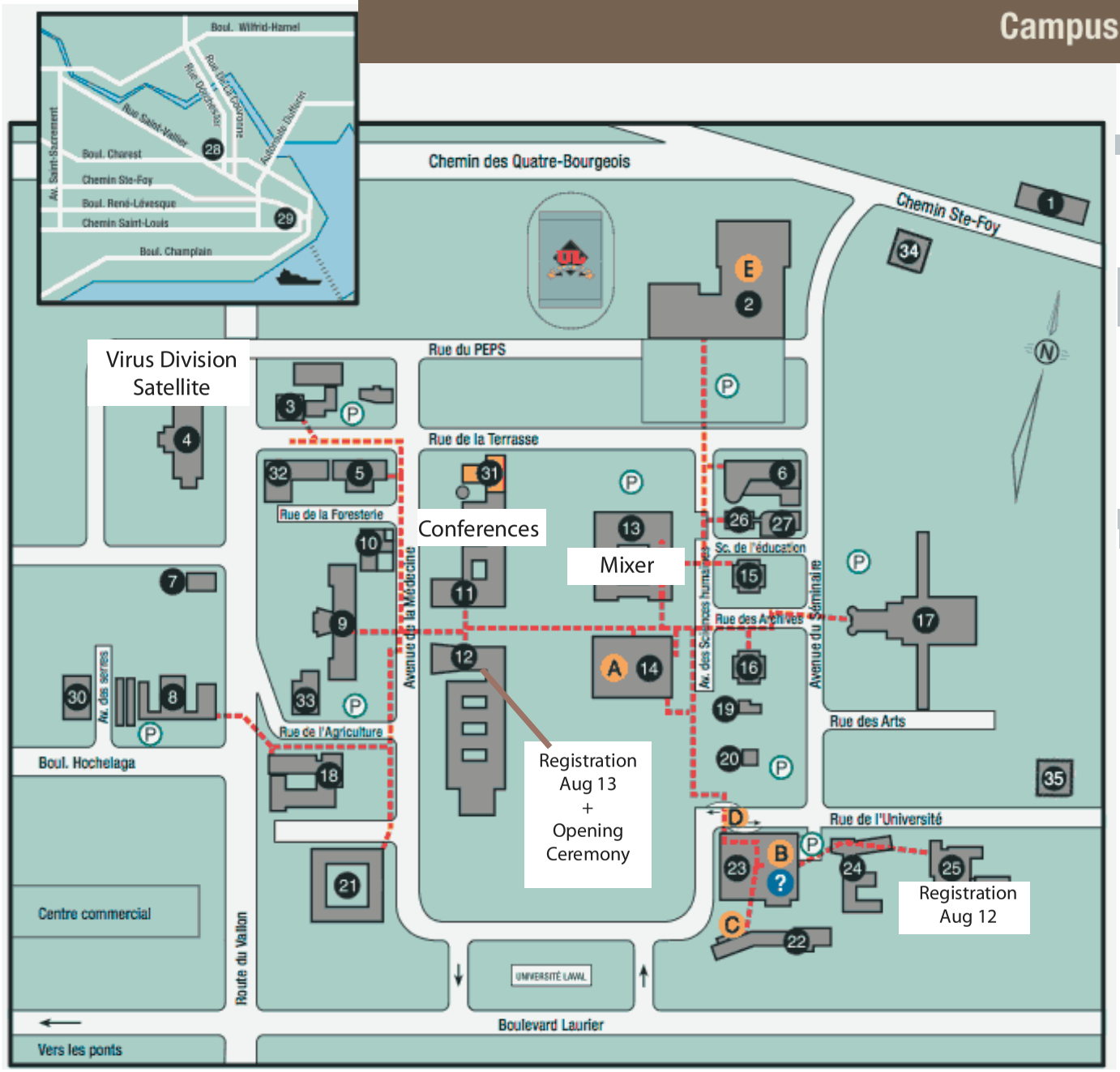
Campus de l'Université Laval



1 Pavillon de l'Est
2 Pavillon de l'éducation physique et des sports (PEPS)
3 Pavillon de Médecine dentaire
4 Centre de foresterie des Laurentides
5 Pavillon Abitibi-Price
6 Pavillon Palasis-Prince
7 Maison Omer-Gingras
8 Pavillon des services
9 Pavillon Ferdinand-Vandry
10 Pavillon Charles-Eugène-Marchand
11 Pavillon Alexandre-Vachon
12 Pavillon Adrien-Pouliot
13 Pavillon Charles-De Koninck
14 Pavillon Jean-Charles-Bonenfant
15 Pavillon des Sciences de l'éducation
16 Pavillon Félix-Antoine-Savard
17 Pavillon Louis-Jacques-Casault
18 Pavillon Paul-Comtois
19 Maison Eugène-Roberge
20 Maison Marie-Sirois
21 Pavillon Agathe-Lacerte
22 Pavillon Ernest-Lemieux
23 Pavillons Alphonse-Desjardins et Maurice-Pollack
24 Pavillon H.-Biermans-L.-Moraud
25 Pavillon Alphonse-Marie-Parent
26 Pavillon J.-A.-De Sève
27 Pavillon La Laurentienne
28 Édifice La Fabrique
29 Édifice du Vieux-Séminaire-de-Québec
30 Pavillon de l'Envirotron
31 Pavillon d'optique-photonique (en construction)
32 Pavillon Gene H. Kruger
33 Édifice logeant Héma-Québec
34 Maison Michael-John-Brophy
35 Pavillon Gérard-Bisaillon (centrale d'énergie)

Services
A Bibliothèque
B Caisse populaire Desjardins, guichet automatique
C Sécurité-stationnement
D Arrêt métrobus (800-801)
E Activités sportives

Résidences
21 Pavillon Agathe-Lacerte
22 Pavillon Ernest-Lemieux
23 Pavillons Alphonse-Desjardins et Maurice-Pollack
24 Pavillon H.-Biermans-L.-Moraud
25 Pavillon Alphonse-Marie-Parent

UNIVERSITÉ LAVAL

Laval University Campus Map

General Information

AUGUST 12th

REGISTRATION - from 1:00 pm to 7:00 pm
WHERE: Pavillon Moreau, Main Entrance (see #24 on the MAP)
Laval University Residences Services
Université Laval, Québec
Phone number: (418) 656-5632
email: hebergement@sres.ulaval.ca

MIXER - from 6:00 pm to 9:00 pm
WHERE: Pavillon Charles-de Koninck, L'ATRIUM (see #13 on the MAP)

AUGUST 13th

REGISTRATION - from 7:30 am to 12:30pm
WHERE: Pavillon Adrien-Pouliot, Room 1112 (see #12 on the MAP)

The REGISTRATION DESK will move to the Pavillon Alexandre-Vachon (see # 11 on the MAP) in the afternoon (where sessions will be held).

AUGUST 14th

5 KM RUN / WALK - NEW DEPARTURE TIME: 6:30 am
WHERE: Pavillon PEPS (see #2 on the MAP)
Numbers will be provided to you at the registration desk on August 12th.

You can get ready for your run at the PEPS, which is located at a 15 minutes walk from the starting line. If you want to leave your personal belongings there, PLEASE BRING YOUR OWN PADLOCK, as only a few padlocks will be available on site.

A snack will be provided right after the run and you will have access to the PEPS for showering/changing.
Click on this link to see the run route.

EXCURSION -
MEETING TIME: 12:30 pm - DEPARTURE TIME: 12:45 pm
MEETING POINT: Pavillon Alexandre-Vachon, Laval University
A bus will pick you up in front of the building and a LUNCH BOX will be provided on your way to the cruise.

1) BOAT CRUISE on the M/V Louis Jolliet
From 2:00 pm to 3:30 pm

2) WATERFALLS
After the cruise, we will make a stop at the Montmorency Waterfalls
From 4:00pm to 5:00pm

**After visiting the Montmorency Waterfalls, buses will drive directly to the Mont Sainte-Anne for the BBQ Evening.

BBQ AT MONT SAINTE-ANNE
WHERE: Chalet du Sommet (Mont Sainte-Anne)
From 5:00 pm until 12:00 am

IF YOU ARE NOT GOING TO THE EXCURSION, BUT ONLY TO THE BBQ:
Shuttles will pick you up at Laval University at 4:15 pm.
MEETING POINT: Pavillon Alphonse-Marie-Parent (#25 on the MAP) (Residences)
DEPARTURE TIME: 4:15 pm

After the BBQ, progressive departures will be scheduled. On the way back to Quebec City, buses will be making several stops: Hotel Le Delta, Hotel Universel, Hotel Le Classique, Wilfrid & Lindbergh.

IF YOU ARE GOING BY CAR TO THE BBQ:
From Laval University:
Take Highway Robert Bourrassa (#740 North), and Highway 40 East (Autoroute de la Capitale), then Route 138 East, following indications for Sainte-Anne-de-Beaupré and Mont-Sainte-Anne. In Beaupré, follow Route 360 to Mont-Sainte-Anne.

From Downtown Québec:
Take Highway Dufferin-Montmorency (#440 East) and Route 138 East, following indications for Sainte-Anne-de-Beaupré and Mont-Sainte-Anne. In Beaupré, follow Route 360 to Mont-Sainte-Anne.

You will find a MAP of the area at this address:
http://www.tourisme-hebergement-ski-mont-sainte-anne-quebec.com/images/Carte01.jpg

INTERNET ACCESS

Room 2820 will be open as a free "Internet Cafe". Only one computer will be available but there will be extra ports to connect your laptop. Please bring YOUR OWN CABLE.

POSTER PRESENTATION

Your poster cannot exceed 3' X 4' (91 cm high by 1,2 meter wide).

Poster Session 1: Posters (Bacteria, Microbial Control and Virus I) are to be set up on Monday morning and taken down by Tuesday afternoon by 5:00 pm.
Poster Session 2: Posters (Fungi, Nematodes, Microsporidia and Viruses II) are to be set up early Wednesday afternoon, and taken down Thursday before the Banquet.

WHERE TO EAT

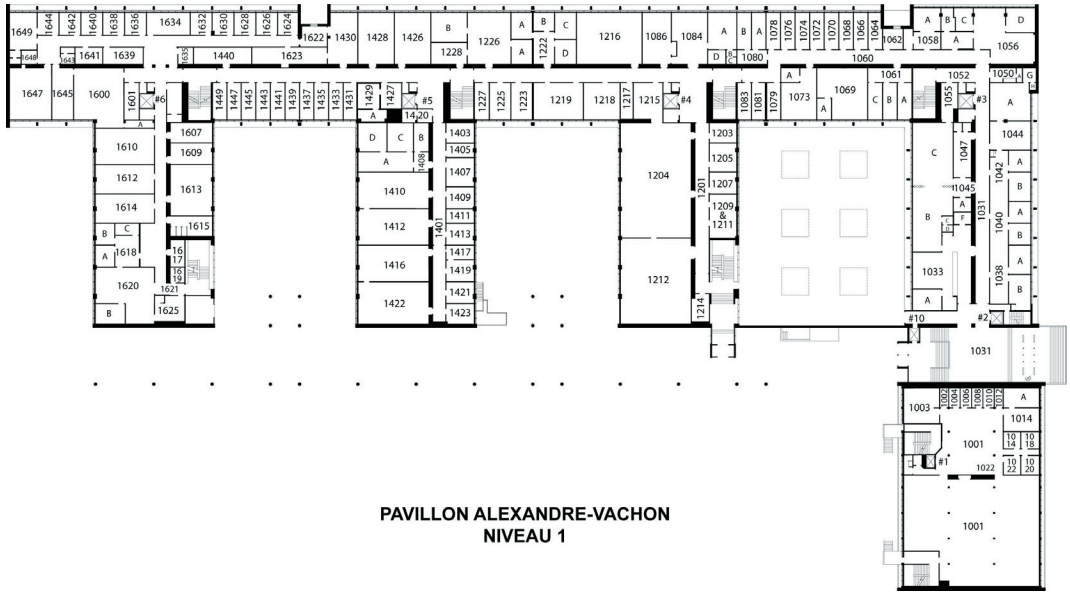
Meals on Campus are available at the conference building cafeteria, Pavillon Vachon (# 11 on the MAP) and in buildings nearby:
Pavillon Charles-De Koninck (#13 on the MAP)
Pavillon Alphonse-Desjardins (#23 on the MAP)

Off Campus:
Place Ste-Foy (10 minutes walk from the Pavillon Vachon)

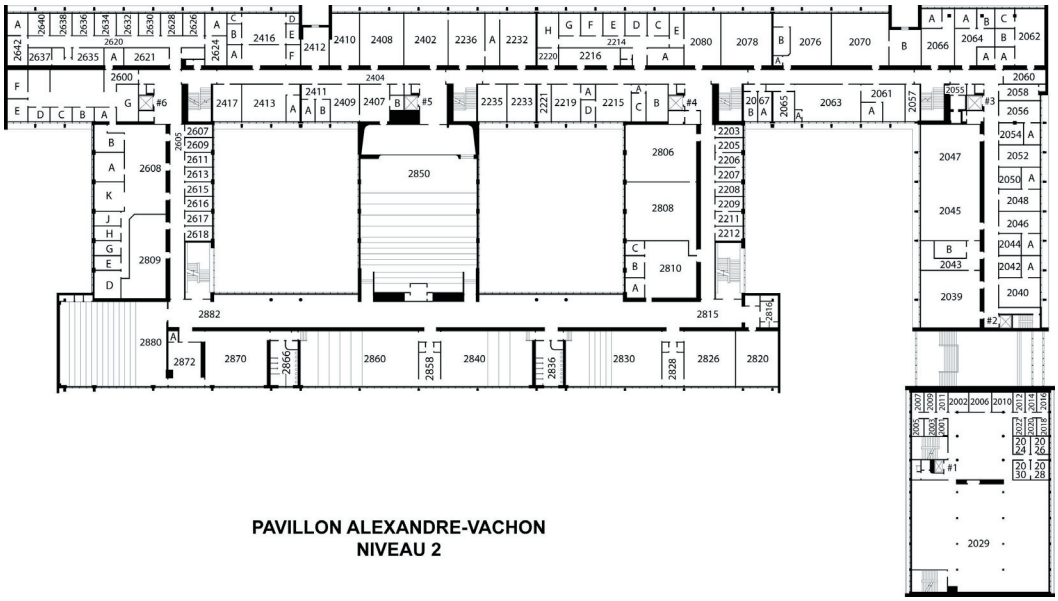
FOOD RESTRICTIONS

If you have any FOOD RESTRICTION, please go to the Registration Desk, so we can make sure we satisfy your needs as best as possible.

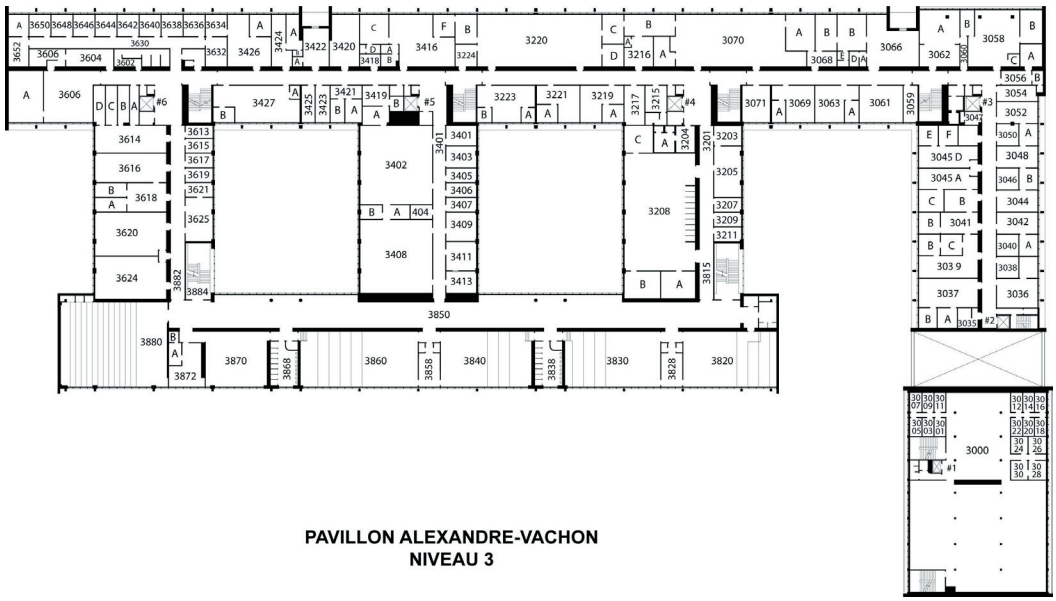
Meetings Building: Pavillon Alexandre-Vachon



PAVILLON ALEXANDRE-VACHON
NIVEAU 1



PAVILLON ALEXANDRE-VACHON
NIVEAU 2



PAVILLON ALEXANDRE-VACHON
NIVEAU 3

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Secretary-treasurer: Zhengzhi Li.
Member at Large: Richard Meadow
Member at Large: Sean Moore

Division of Microsporidia
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Chair-Elect: David Oi
Secretary/Treasurer: Dörte Goertz
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Rollie Clem (USA) (2006-2008)

Student representative:
Jondavid de Jong (2005-2007)
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SIP COMMITTEES

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Jean-Louis Schwartz, Université de Montréal, co-chair
Bruce Broadbent, Agriculture and Agri-Food Canada
Jacques Brodeur, Université de Montréal
Jean-Charles Côté, Agriculture and Agri-Food Canada
Chris Lucarotti, Natural Resources Canada

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SIP Division chairs:
Bacteria - Christina Nielsen-LeRoux
Fungi - Fernando E. Vega
Microbial Control - Michael Brownbridge
Microsporidia - Regina Kleespies
Nematodes - David Shapiro-Ilan
Viruses - Johannes Jehle

Social program committee
Raynald Laprade, Université de Montréal, chair
Roslyn Cabot, Montreal
Conrad Cloutier, Université Laval
Jean-Louis Schwartz, Université de Montréal

PROGRAM
2007

Note: Only presenters' names are given here. For full listing of authors and affiliations, please refer to the Abstracts.

Start times for Symposia are fixed. Start times for individual speakers within Symposia are only estimates. It is up to the conveners of each symposium to determine and regulate lengths of individual presentations. Discussion periods may follow if time permits

SUNDAY, AUGUST 12TH

08:30 - 17:00 SIP Council Meeting, Room VCH-1039C
13:30 - 19:00 Registration
18:00 - 21:00 Mixer

MONDAY, AUGUST 13TH

7:00 - 9:00 Registration Pavillon Vachon

Monday, 8:30 - 10:00, Room PLT-1112
Opening Ceremony and Founders' Lecture

Opening Ceremony and Founders' Lecture
Jean-Louis Schwartz, Chair, Organizing Committee
Wendy Gelernter, President, SIP

Founders' Memorial Lecture: Albert K. Sparks: A Pioneer and Visionary in Non-Insect Invertebrate Pathology
Dudley Pinnock, Chair, Founders' Lecture Committee
Dr. Albert K. Sparks, Honoree
Dr. Frank Morado, Lecturer

10:00 - 10:30 COFFEE BREAK

Monday, 10:30 - 12:30, Room PLT-1112
Plenary Lectures

10:30 **1 Looking back: 40 years of SIP** Elizabeth W. Davidson, School of Life Sciences, Arizona State University, Tempe, AZ
11:30 **2 Chemical ecology and invertebrate pathology: Do sub-lethal pathogenic infections affect chemically mediated behaviors?** Jeremy McNeil & Jacques Brodeur

12:30 - 14:00 LUNCH

Bacterial Division Symposium Monday 14:00 - 16:00, Room VCH-2850
Mode of actions of toxins
Conveners: Jeroen Van Rie & Juan Ferré

14:00 **3 New Insight into the Mode of Action of Bacillus thuringiensis Cry Toxin: Cell Death by cAMP** Lee Bulla, Biological Targets, Inc., Pilot Point, TX and The University of Texas at Dallas, Richardson, TX
14:30 **4 The pre-pore oligomer is an obligate intermediate in the cell death induced by Bacillus thuringiensis Cry1Ab toxin in insect larvae.** Mario Soberon, Instituto de Biotecnologia UNAM
15:00 **5 A Critique of Current Models for the Mode of Action of BT Toxins** Donald H. Dean, Department of Biochemistry, The Ohio State University, Columbus, Ohio
15:30 **6 Thinking outside the box for the design of Bt products: can we take advantage of their target cells environment and physiological response to aggression?** Jean-Louis Schwartz, Université de Montréal

Virus Division Symposium I Monday 14:00 - 16:00, Room VCH-2880
Insect cells and baculoviruses 'Pas de Deux' - A Symposium in honor of Bob Granados
Convener: Just Vlak

14:00 **Introduction** Just Vlak
14:05 **7 Developments and significance in insect cell culture** Dwight Lynn, INSell Consulting, 247 Lynch Rd, Newcastle, ME 04553
14:30 **8 The Peritrophic membrane and the role of enhancins** Ping Wang, Department of Entomology, Cornell University, NYSAES, Geneva, NY 14456
14:55 **9 Viral entry in insect cell systems** Gary W. Blissard, Cornell University, Ithaca
15:20 **10 Contributions to virology by Robert R. Granados: reflections by a colleague and friend** Brian A. Federici, University of California
15:45 **10,1 Laudatio.** Johannes A. Jehle, Chair Virus Division

IFENSB Monday 14:00 - 16:00, Room VCH-3860
Session I: Symbiosis
Moderator: Mary Barbercheck

14:00 **11 Molecular aspects of Xenorhabdus nematophila-Steinernema carpocapsae association** Heidi Goodrich-Blair, Department of Bacteriology, University of Wisconsin-Madison
14:20 **12 The ins and outs of Photorhabdus luminescens transmission by Heterorhabditis bacteriophora.** Todd Ciche, Michigan State University
14:40 **13 The biosynthesis of stilbene in Photorhabdus luminescens** David Clarke, University College Cork
15:00 **14 Flexible gene pool in genomes of the entomopathogenic bacteria, Photorhabdus and Xenorhabdus.** Sophie Gaudriault, INRA-Université Montpellier II, France
15:20 **15 EPN Phylogeny and Evolution.** Byron Adams, Brigham Young University

Contributed Papers Monday 14:00 - 16:00, Room VCH-3880
Fungi 1
Chair: Fernando E. Vega

14:00 **16 Effect of esterase over-expression on the virulence of Beauveria bassiana infecting the coffee berry borer** Carmenza E. Gongora B., Department of Entomology. CENICAFE-FNC. Colombia
14:15 **STU 17 Interactions of two natural enemies of Tetranychus evansi, the fungal pathogen Neozygites floridana (Zygomycetes: Entomophthorales) and the predatory mite, Phytoseiulus longipes (Acari: Phytoseiidae)** Vitalis Wekesa, Department of Entomology, Plant Pathology and Agricultural Zoology, ESALQ / University of São Paolo
14:30 **18 Detection and avoidance of Beauveria bassiana by seven spot ladybirds, Coccinella septempunctata** Emma Ormond, Anglia Ruskin University

14:45 **STU** **19** **Molecular characterisation of Beauveria bassiana isolates obtained from semi-field arenas of overwintering Coccinella septempunctata** Emma Ormond, Anglia Ruskin University

15:00 **20** **Myrmica rubra defense against entomopathogenic fungi** Eleanor Groden, School of Integrative Biology and Ecology, University of Maine

15:15 **21** **The invasive coccinellid Harmonia axyridis as an intra-guild predator of the aphid-specific fungus Pandora neoaphidis** Helen Roy, Anglia Ruskin University, Centre for Ecology and Hydrology

15:30 **22** **Exposure to Beauveria bassiana reduces the fecundity of Harmonia axyridis** Helen Roy, Anglia Ruskin University / Centre for Ecology and Hydrology, UK

15:45 **23** **A tale of two Continents: Cordyceps in ants** David Hughes, University of Copenhagen

16:00 - 16:30 COFFEE BREAK

Monday, 16:30 - 18:30, Pavillon Vachon, 2nd Floor

POSTER SESSION I

Bacteria

B-01 **Three Bt-resistant populations of Plutella xylostella with diverse phenotypes share a common resistance locus.** Ali Sayyed, School of Life Sciences, University of Sussex, Falmer, Brighton, East Sussex BN1 9QG, United Kindom

B-02 **Study of the mechanism of resistance to Bacillus thuringiensis Cry3A toxin in a natural population of the leaf beetle, Chrysomela tremulae (Coleoptera: Chrysomelidae)** Munster, Manuella Van Institut National de la Recherche Agronomique

B-03 **Characterization of intracellular signaling in mosquitoes in response to Bacillus thuringiensis subspecies israelensis toxins** Angeles Cancino-Rodezno Instituto de Biotecnologia UNAM, Mexico

B-04 STU **Involvement of a Colorado potato beetle membrane associated metalloprotease on Cry3Aa Bacillus thuringiensis mode of action** Camila Ochoa-Campuzano, Departamento de Genética. Universidad de Valencia. Spain

B-05 **Ultrastructure of Culex quinquefasciatus midgut cells from susceptible and Bacillus sphaericus-resistant larvae: morphology and cytopathological effects** Helena Neves Lobo Silva-Filha, Maria Department of Entomology, Centro de Pesquisas Aggeu Magalhães-Fundação Oswaldo Cruz

B-06 **Immunolocalization of the gypsy moth Bacillus thuringiensis toxin receptor by electron microscopy** Algimantas Valaitis USDA Forest Service

B-07 **Overcoming microbial insecticide resistance in blackfly larvae by combinations of them.** Guy Charpentier Département de chimie-biologie, Université du Québec à Trois-Rivières

B-08 **Agricultural BioTech Regulatory Network** Susan MacIntosh MacIntosh & Associates, Inc., Saint Paul, MN, USA

B-09 **Why are Bacillus thuringiensis formulations ineffective against Spodoptera litura feeding on strawberry leaves?** Takeshi Suzuki Tokyo University of Agriculture and Technology

B-10 **Entomopathogenic and non pathogenic bacterial antigens affect Malacosoma disstria (Lepidoptera: Lasiocampidae) larval hemocytes in vivo and in vitro** Paschalis Giannoulis McGill University, Department of Natural Resource Sciences, QC H9X 3V9, Canada

B-11 **Conjugative relationship among Bacillus thuringiensis and Bacillus cereus strainsSantos, CA; Vilas-Bôas, GT; Arantes, OMN Centro de Ciências Biológicas, Departamento de Biologia Geral, Universidade Estadual de Londrina/PR, Brazil** M Nagy ARANTES, Olivia Centro de Ciências Biológicas, Departamento de Biologia Geral, Universidade Estadual de Londrina

B-12 **Isolation of mosquitocidal and non-mosquitocidal Bacillus sphaericus** Hyun-Woo Park Public Health Entomology Center, Florida A & M University

B-13 **Isolation and characterization of a novel Bacillus thuringiensis strain expressing a novel crystal protein with cytocidal activity against human cancer cells** Jean-Charles Côté Agriculture and Agri-Food Canada

B-14 **Systemic use of Bacillus thuringiensis, a new alternative for the control of insects pest** Erica Martins Embrapa Recursos Genéticos e Biotecnologia

B-15 **Production and efficacy of an experimental tablet formulation for Aedes aegypti control using a Bacillus thuringiensis var israelensis asporogenic mutant strain** Sergio Orduz Corporación para Investigaciones Biológicas

B-16 **Characterization of sporulation histidine kinases from Bacillus thuringiensis** Maria Islas-Osuna Centro de Investigación en Alimentación y Desarrollo, A.C.

B-17 **Functional studies of the Insecticidal Toxin Complexes of Photorhabdus luminescens and Yersinia** Michelle Hares University of Exeter in Cornwall

B-18 **Genetic variation of Helicoverpa armigera populations around the cotton growing area in southern Spain as revealed by amplified fragment length polymorphism (AFLP)** Baltasar Escriche University of Valencia

B-19 **16S ribosomal RNA based assessment of the taxonomic position of Rickettsiella melolonthae** Regina G Kleespies Federal Biological Research Centre for Agriculture and Forestry (BBA)

B-20 **Molecular characterization of a novel mosquitocidal crystal protein, Cry50A, from a Japanese isolate of Bacillus thuringiensis serovar sotto strain** Akira Ohgushi Department of Chemistry, Faculty of Science, Kyushu University, Fukuoka, Japan

B-21 **Cloning and expression of the potential receptor of Bacillus sphaericus binary toxin in Anopheles gambiae** Helena Neves Lobo Silva-Filha, Maria Department of Entomology,Centro de Pesquisas Aggeu Magalhães-Fundação Oswaldo Cruz

B-22 **Mutagenic nnalysis of loops in the receptor binding domain of Bacillus thuringiensis cry11Ba toxin** Supaporn Likitvivatanavong University of California Riverside

B-23 **Use of Green Fluorescent Protein to monitor the pathology of a unique Yersinia sp,. able to kill many insect species within 72 hours.** Mark Hurst AgResearch, NZ

B-24 **Phylogeny and host range testing of a a novel entomopathogenic species of Enterobacteriaceae,** Mark Hurst, Agresearch

B-25 **Identification of three Zwittermicin A biosynthesis-related genes from Bacillus thuringiensis subsp. kurstaki strain YBT-1520** Ming Sun State Key Laboratory of Agricultural Microbiology, College of Life Science & Technology, Huazhon

B-26 STU **A 106-kDa aminopeptidase is a putative receptor for Bacillus thuringiensis Cry11Ba toxin in the mosquito Anopheles gambiae** Rui Zhang Departments of Entomology, University of Georgia, Athens, GA 30602.

B-27 **Identification of Aedes aegypti alkaline phosphatase receptors of Bacillus thuringiensis subsp. isrealensis Cry 11A toxin** Jianwu Chen Department of Cell Biology and Neurosciences, University of California, Riverside, CA 92521

B-28 **Identification and analysis of Clostridium bifermentans mosquitocidal proteins** Ravneet Sandhu University of California Riverside

B-29 **Identification of a cadherin in Anopheles gambiae larvae as a putative receptor for Bacillus thuringiensis israelensis Cry4Ba toxin.** Gang Hua Departments of Entomology , University of Georgia, Athens, GA 30602.

B-30 STU **A proteomic approach to the identification of Cry4Ba binding proteins in midgut membranes from Aedes aegypti** Krishna Bayyareddy Departments of Entomology University of Georgia, Athens, GA30602.

B-31 **Sequence diversity of the Bacillus thuringiensis flagellin (H-antigen, Hag) protein - Comparison with H-serotype diversity** Jean-Charles Côté Agriculture and Agri-Food Canada

B-32 **Unusual organization associated to a tandem of IS231 may yield two peculiar cloverleaf secondary structures** Jean-Charles Côté Agriculture and Agri-Food Canada

B-33 **Lipid specificity of the Cyt1A/membrane interaction** Peter Butko University of Southern Mississippi

B-34 STU **Mega assemblage of a mammalian cell-targeting and pore-forming toxin parasporin-2 from Bacillus thuringiensis** Hiroyasu Shimada Department of Chemistry, Faculty of Science, Kyushu University, Fukuoka 812-8581, Japan

B-35 **Functional genomics of Photorhabdus asymbiotica. Rapid Virulence Annotation (RVA) of pathogen genomes using invertebrate models.** Nick Waterfield University of Bath

B-36 STU **Susceptibility of Spodoptera exigua to 9 toxins from Bacillus thuringiensis** Patricia Hernandez-Martinez Departmento de Genetica, Universitat de Valencia, Dr. Moliner 50, 46100-Burjassot (Valencia), S

B-37 STU **The metabolic regulation of thuringiensin biosynthesis by Bacillus thuringiensis strain YBT-1532** Wang Zhi Guo Chengliang, Chen Shouwen, Ruan Lifang, Sun Ming and Yu Ziniu

Microbial Control

MC-01 **Development of a repository for baculoviruses (a Baculobank) in Canada** Renée Lapointe Sylvar Technologies Inc.

MC-02 **Susceptibility of Cydia pomonella to mixed preparation of granulosis virus and Bacillus thuringiensis** Liga Jankevica Department of Experimental Entomology, Institute of Biology, University of Latvia, Miera iela 3,

MC-03 **Semiochemical autodissemination of tortricid viruses in the orchard** Winstanley Doreeen University of Warwick

MC-04 **Antifeeding toxin or just bad taste?** Sean Marshall Biocontrol and Biosecurity, AgResearch, Private Bag 4749, Christchurch 8140, New Zealand

MC-05 **Proteinase activities and proteolytical processing of the B.t.-corn-toxin Cry3Bb1 in the midgut of Western Corn Rootworm (Diabrotica virgifera virgifera)** Renate Kaiser-Alexnat Federal Biological Research Centre for Agriculture and Forestry (BBA), Institute for Biological

MC-06 **A peptide derived from Manduca sexta Bt-R1a cadherin enhances activity of commercial Bt formulation on Bt-susceptible and Bt-resistant insects** Mohd Abdullah InsectiGen, Inc., 425 River Road, Athens, GA 30602

MC-07 STU **Efficacy of Beauveria bassiana (Bals.) Vuill. against the tarnished plant bug, Lygus lineolaris L., in strawberry field** Rachid Sabbahi INRS-Institut Armand-Frappier

MC-08 **Effect of soil management on naturally occurring entomopathogenic fungi during the transition to an organic farming system** Mary Barbercheck Department of Entomology, Pennsylvania State University

MC-09 STU **Pathogenicity of Metarhizium anisopliae expressing the scorpion toxin (AaIT) against the coffee berry borer, Hypothenemus hampei (Coleoptera: Curculionidae)** Monica Pava-Ripoll Department of Entomology, University of Maryland, College Park, Maryland 20742 USA

MC-10 **Effect of amber disease on regulation of midgut proteases in the New Zealand grass grub Costelytra zealandica** Sean Marshall Biocontrol and Biosecurity, AgResearch, Private Bag 4749,Christchurch 8140, New Zealand

MC-11 **Susceptibility of Rhagoletis indifferens (Diptera: Tephritidae) larvae and pupae to infection by Beauveria bassiana** Joan Cossentine Agriculture and Agri-Food Canada

MC-12 **Quantitative expression of delta-endotoxin protein in HD-1-S-2005, the proposed new international reference standard for Bacillus thuringiensis** Larry Gringorten Canadian Forest Service, Great Lakes Forestry Centre, Sault Ste. Marie, Ontario P6A 2E5, Canada

MC-13 **To Bt or not to Bt: do we need an international reference standard? Pros, cons and future directions** Larry Gringorten Canadian Forest Service, Sault Ste. Marie, Ontario P6A 2E5, Canada

Virus I

V-01 **Commensal and mutualistic relationships of reoviruses with their parasitoid wasp hosts** Sylvaine RENAULT LEPG, FRE CNRS 2969, UFR Sciences et Techniques, Université François Rabelais, Tours, France

V-02 **Evolution of ascovirus from baculovirus-a hypothesis** Xiao-Wen Cheng Department of Microbiology, Miami University, Oxford, Ohio 45056 USA

V-03 **Is the presence of persistent baculovirus infections linked to insect immunity?** Elizabeth Kemp Great Lakes Forestry Centre, Canadian Forest Service, Sault Ste. Marie, ON, Canada/ Biology Depa

V-04 **Isolation and molecular characterization of a nucleopolyhedrovirus, granulovirus and cypovirus from populations of the western spruce budworm Choristoneura occidentalis (Lepidoptera: Tortricidae) in British Columbia, Canada.** Christopher Lucarotti Natural Resources Canada, Canadian Forest Service-Atlantic Forestry Centre, P.O. Box 4000, Frede

V-05 **Selection of a new virus isolate to control CpGV resistant** Martin Andermatt Andermatt Biocontrol AG, Stahlermatten 6, 6146 Grossdietwil, Switzerland

V-06 STU **Fast- and slow-killing genotypic variants in a Dutch isolate of Adoxophyes orana nucleopolyhedrovirus** Maho Takahashi Department of Plant Protection, Tokyo University of Agriculture and Technology, Fuchu, Japan

V-07 STU **Combined use of CpGV granulovirus and Trichogramma wasps against codling moth, an apple pest** Olivier Morisset, Université du Québec à Montréal, C.P. 8888, Succursale Centre ville, Montréal (Québec) H3C 3P8

V-08 **Recombinant Sindbis viruses that regulate apoptosis in the C6/36 Aedes albopictus cell line** Rollie Clem Kansas State University

V-09 STU **Expression analysis of polydnavirus rep genes in Choristoneura fumiferana larvae parasitized by Tranosema rostrale** Asieh Rasoolizadeh Département de biologie, Université Laval, Québec, QC, Canada and Natural Resources Canada

V-10 **Campoletis sonorensis polydnavirus ankyrin and cys-motif genes affect on baculovirus replication in H. virescens and H. zea.** Bruce Webb University of Kentucky

V-11 **Identification and characterization of novel RNA sequences associated with late male-killing in the oriental tea tortrix, Homona magnanima (Lepidoptera: Tortricidae)** Kazuko Nakanishi Department of Bioregulation and Biointeraction, Graduate School of Agriculture, Tokyo University

V-12 **The role of innate immunity of gypsy moth under exogenous infection by nucleopolyhedrovirus.** Viatcheslav Martemyanov Institute of systematics and ecology of animals SB RAS

V-13 **Apoptosis is induced in haemolymph and fat body of Spodoptera exigua larvae upon oral inoculation with Spodoptera litura nucleopolyhedrovirus** Kai Yang 1 State Key Laboratory of Biocontrol, Sun Yat-sen University, Guangzhou 510275, China

V-14 **Impact of enhancin genes on potency of LdMNPV in oak-fed gypsy moths** Merideth Humphries Department of Entomology, Penn State University, University Park, PA 16802 USA

V-15 **Functional analysis and baculovirus expression of serpins from the molting fluid of the spruce budworm, Choristoneura fumiferana** Yiping Zheng Great Lakes Forestry Centre, 1219 Queen Street East, Sault Ste. Marie, ON, Canada P6A 2E5

V-16 **Effects of Choristoneura fumiferana defective nucleopolyhedrovirus spindlin on viral infectivity and the hosts immune genes** Cailing Liu Great Lakes Forestry Center, Sault Ste. Marie, Ontario, Canada P6A 2E5

V-17 **Importance of peroral infection factors (pif and pif-2) in the interactions between genotypes of Spodoptera frugiperda multinucleopolyhedrovirus (SfMNPV).** Gabriel Clavijo Laboratorio de Entomología Agrícola y Patología de insectos, Departamento de Producción Agraria,

V-18 **Detoxification enzymes of Spodoptera littoralis Boisd. infected with a baculovirus** Oktay Gurkan Ankara University -Turkey

V-19 STU **Characterization of a baculovirus isolated from a diseased larva of Adoxophyes honmai in Japan** Rie Ukuda Tokyo University of Agriculture and Technology

V-20 **Pathogenicity and viral multiplication of Xestia c-nigrum granulovirus in larvae of Mythimna separata (Lepidoptera: Noctuidae)** Shigeyuki Mukawa Insect Pest Management Research Team, National Agricultural Research Center.

V-21 **Vertical transmission of ChfuGV into spruce budworm Choristoneura fumiferana sub-lethally infected larvae.** Rosa Maria de Moraes Institut Armand-Frappier. 531, Boulevard. des Prairies, Laval, Québec, Canada. H7V 1B7.

V-22 **Relative frequency of HaSNPV and HzSNPV nucleopolyhedrovirus after multiplication in different hosts** Salvador Herrero Department of Genetics, University of Valencia, Spain

V-23 **Modulation of host gene expression in a spruce budworm cell line infected by wild type or recombinant Choristoneura fumiferana nucleopolyhedrovirus** Dayu Zhang Department of Biology, Indiana University, 1001 E. 3rd St. Bloomington, IN USA 47405

V-24 **Expression of foreign protein by a polyhedrin-positive, cathepsin null recombinant baculovirus.** Olga Lihoradova Institute of Chemistry of Plant Substances, Tashkent, Uzbekistan

V-25 STU **Construction of advanced baculovirus expression vector for generating high-throughput recombinant proteins** Yang-Su Kim Department of Agricultural Biotechnology, Seoul National University, Seoul, Korea

Contributed papers Monday 16:30 – 18:00, Room VCH-2850
Microsporidia
Chair: James Becnel

16:30 **24 Life cycle characteristics of microsporidia influence their transmission in a lepidopteran host** Dörte Goertz, University of Natural Resources and Applied Life Sciences, Vienna
16:45 **25 Multiple microsporidia species detection in Agrilus anxius, a congener to emerald ash borer, Agrilus planipennis: - possible biological control agent** George Kyei-Poku, Natural Resources Canada, Canadian Forestry Service, Great Lakes Forestry Centre
17:00 **27 Rapid molecular diagnosis of nosema disease in honeybee** Wei-Fone Huang, Department of Entomology, National Taiwan University, Taipei 106, Taiwan
17:15 **STU** **28 In vitro propagation of a microsporidian isolate (Nosema sp.) from yellow butterfly, Eurema blanda** Yi-Chun Tsai, Department of Entomology, National Taiwan University, Taipei 106, Taiwan
17:30 **28,05 Phylogeny and classification of the phylum Microsporidia** Charles R. Vossbrinck, Connecticut Agricultural Experiment Station, 123 Huntington Street, New Haven Connecticut, 06511, USA

Monday, 20:00-21:00, Room VCH-2850
Bacteria Division Business Meeting

Bacteria Division Workshop Monday, 21:00-22:00, Room VCH-2850
So Many Strains, so Few Products! Opportunities and Constraints to Commercial Development of New Bt Products
Conveners: Ole Skovmand & Trevor Jacson

21:00 **28,1 The regulatory hurdle.** Brian Belliveau
21:15 **28,2 Why companies don't pick up new strains.** Prem Warrior
21:30 **28,3 Natural variation in Bt Cry toxins.** Neil Crickmore
21:45 **28,4 Criteria for selection and use of native Bt strains.** Rose Monnerat

Monday, 20:00-22:00, Room VCH-3870
Fungal Division Business Meeting

Microsporidia Division Business Meeting and Workshop, Monday 20:00 - 22:00, Room VCH-2880
Workshop: "Methods of field inoculation with microsporidia", A Roundtable Discussion. Organizer: David Oi

Jimmy Becnel (Mosquitoes), Lee Solter (Gypsy Moth), Denny Buck (Black Vine Weevil) and David Oi (Fire Ants)

Nematode Division Business Meeting and Workshop, Monday 20:00-22:00, Room VCH 3860
Workshop: Nematode Phylogeny and Systematics
Convener: Patricia Stock

Virus Division Business Meeting and Workshop, Monday 20:00-22:00, Room VCH-3880
Workshop "Polydnavirus Phylogeny and Taxonomy"
Organizer: Michel Cusson

Features of polydnavirus genome organization and their phylogenetic implications. Bruce Webb
Polydnavirus taxonomy and the ICTV. Peter Krell

TUESDAY, AUGUST 14TH

5k SIP Run/Walk, Departure Time: 6:30 -

Contributed Papers Tuesday 8:00 - 10:00, Room VCH-2850
Microbial Control 1
Chair: Michael Brownbridge

08:00 **29 Efficacy of mixtures of Beauveria bassiana strains in the control of coffee berry borer, under laboratory and field conditions** Carmenza E. Gongora B., Department of Entomology. CENICAFE-FNC. Colombia.
08:15 **STU** **30 Differential gene expression by Metarhizium anisopliae grown on plant root exudate** Monica Pava-Ripoll, Department of Entomology, University of Maryland
08:30 **STU** **31 Biological control of Hoplia philanthus (Coleptera: Scarabaeidae) using entomopathogenic nematodes.** Biswo Adhikari, Brigham Young University
08:45 **STU** **32 Synergistic interaction in white grubs control** Anuar Morales, Cornell University
09:00 **33 Pink Bollworm Resistance to Bt Cotton: Still Rare After All These Years** Bruce Tabashnik, Department of Entomology, University of Arizona, Tucson AZ 85721
09:15 **34 Fungal biopesticides for sucking pest management in Australian broadacre crops.** Caroline Hauxwell, Department of Primary Industries & Fisheries, Queensland, Australia
09:30 **35 Use of Beauveria bassiana and imidacloprid for control of emerald ash borer in an ash nursery** John Vandenberg, USDA ARS, U.S. Plant, Soil & Nutrition Lab., Tower Road, Ithaca, NY 14853
09:45 **36 The fascinating true story about the famous Metarhizium anisopliae isolate Ma43, alias ATCC 90448, alias BIPESCO 5, alias F52 alias .....** Jørgen Eilenberg, University of Copenhagen, Faculty of Life Sciences, Department of Ecology, Thorvaldsensvej 40, D

Virus Division Symposium II Tuesday 8:00 - 10:00 , Room VCH-2880
Baculovirus Bounty: A Symposium to honor Loy Volkman
Convener: Linda A. Guarino

08:00 **Introduction** Linda Guarino
08:05 **37 Viruses Insex and the SIP** John P. Burand, University of Massachusetts
08:30 **38 Functional analysis of the interaction between BmNPV ORF8 and its host factors** WonKyung Kang, Mol. Entomol. Lab., RIKEN
08:55 **39 Expanding baculovirus bounty through glycoengineering** Donald Jarvis, University of Wyoming
09:20 **40 Baculovirus replication sites: role of cellular and viral genes** Dagmar Knebel-Moersdorf, Department of Neurology and Center for Biochemistry, University of Cologne, Cologne, Germany
09:55 **40,1 Laudatio.** Johannes A. Jehle, Chair, Virus Division

IFENSB Session II — Tuesday 8:00 - 10:00 , Room VCH-3860

Virulence

Moderator: Lerry Lacey

08:00 **41 The Race to Death: The interplay between the insect immune defenses and the entomopathogenic nematode cuticle in determining host specificity.** Diana Cox-Foster, Penn State University

08:30 **42 Neuroimmunity: insights from the C. elegans pathogenesis model.** Man-Wah Tan, Department of Genetics, and Department of Microbiology and Immunology, Stanford University School

09:00 **43 Exploiting the Photorhabdus genome** Richard Constant, Biological Sciences, University of Exeter, Pernyn, Cornwall, UK

09:30 **44 Virulence of entomopathogenic nematodes and symbiotic bacteria complexes** Parwinder Grewal, The Ohio State University, OARDC

Cross-Divisional Symposium 1 — Tuesday 8:00 - 10:00 , Room VCH-3880

Current situation on the biological control of turfgrass insects

Conveners: Guy Bélair and Julie Dionne

08:00 **45 Entomopathogenic nematodes for pest management in turfgrass: discovery, development, and implementation** Parwinder Grewal

08:20 **46 Fungi for the control of turfgrass insects: an overview,** Rick Brandenburg, Dept. of Entomology, N. C. State Uniuv., Raleigh NC 27695 USA

08:40 **47 Prospects for Managing Turfgrass Insects with Baculoviruses** Callie Prater, North Carolina State University

09:00 **48 Combination of entomopathogenic nematodes with other control agents for the management of white grubs in turfgrass** Albrecht Koppenhöfer, Dept. Entomology, Rutgers University

09:20 **49 Biological control of turfgrass insect pests in Canada current situation** Louis Simard, Horticulture Research and Development Centre, Agriculture and Agri-Food Canada, St-Jean-sur-Rich

10:00 - 10:30 COFFEE BREAK

Contributed Papers — Tuesday 10:30 - 12:30 , Room VCH-2850

Bacteria 1

Chair: Alejandro Bravo

10:30 **50 Death caused by Bacillus thuringiensis is followed by extensive colonization and sporulation in natural and certain atypical hosts** Brian Federici, Department of Entomology, University of California-RIverside, Riverside, CA

10:45 **51 The genomic structure of three Lepidoptera cadherin-like Bacillus thuringiensis related genes** Baltasar Escriche, Genetics Department, University of Valencia, Dr Moliner, 50, 46100, Burjassot, Valencia, Spain

11:00 **STU** **53 Identification and characterization of compounds involved in the stimulation of cry1Aa expression of Bacillus thuringiensis var. kurstaki** Angel Emilio Aceves Diez, Centro de Investigación en Alimentación y Desarrollo A.C

11:15 **STU** **54 The prepore-oligomer is an obligate intermediate in the cell death induced by Bacillus thuringiensis Cry1Ab toxin insect larvae,** Nuria Jiménez

11:30 **STU** **55 Single Mutation in Domain 2 of Cry1Ab Toxin Affects the Insertion of the Toxin into Insect Membranes** Manoj Nair, Biophysics Program and Department of Biochemistry, The Ohio State University, Columbus, Ohio 432

11:45 **56 GFP Expression in wild-type B. thuringiensis strain active to Lepidoptera** Marlene T De-Souza, Cell Biology Department, Brasilia University - Brazil

12:00 **57 Characterization of a new quorum-sensing system in Bacillus thuringiensis** Didier Lereclus, INRA

12:15 **57,5 Production and characterization of Bt Cry1Ac resistance in cotton bollworm, Helicoverpa zea (Boddie)** William Moar, Department of Entomology and Plant Pathology, Auburn University, Auburn AL, USA 36849

Contributed Papers — Tuesday 10:30 - 12:30 , Room VCH-2880

Viruses 1. Viral Ecology and Biocontrol

Chairs: Martin Erlandson & Zhihong (Rose) Hu

10:30 **58 Variation in the prey-processing behavior of insectivorous birds affects NPV transmission in the gypsy moth, Lymantria dispar.** James Reilly, Cornell University

10:45 **59 Host plant-mediated changes to the peritrophic matrix influence baculoviral pathogenesis** Ruth Plymale, Department of Entomology, Cornell University, Ithaca, NY 14853 USA

11:00 **60 Impact of host plants on the peritrophic matrix as a barrier to baculovirus** Kelli Hoover, Penn State University

11:15 **STU** **61 Effects of developmental resistance on LdMNPV pathogenesis in gypsy moth** Jim McNeil, Penn State University

11:30 **STU** **62 Inheritance of field resistance of codling moth against Cydia pomonella granulovirus (CpGV)** Sabine Asser, Laboratory of Biotechnical Crop Protection, Department of Phytopathology, Agricultural Service C

11:45 **STU** **63 On the validity of the independent action hypothesis model for the nucleopolyhedroviruses: can infection with a single virion lead to host mortality?** Mark Zwart, Laboratory of Virology, Wageningen University, The Netherlands

12:00 **64 Is there evidence for selection for resistance to viral disease in cyclic populations of tent caterpillars?** Jenny Cory, 1Laboratory for Molecular Ecology, Great Lakes Forestry Centre, Sault Ste. Marie, ON, P6A 2E5, 2

12:15 **65 The use of Baculovirus to control fall armyworm, Spodoptera frugiperda, in Brazil.** Fernando Embrapa Valicente

Contributed Papers — Tuesday 10:30 - 12:30 , Room VCH-3860

Nematodes

Chair: TBD

10:30 **66 Biochemical and molecular characterization of symbiotic bacteria of four Steinernema from Costa Rica, S. costaricense n.sp.(CR9), S. puntauvense n. sp. (Li6), S. websterii (CR5) and Steinernema sp. (T4) (Rhabditida: Steinernematidae)** Lorena Uribe Lorio, Centro de Investigación en Biología Celular y Molecular, Universidad de Costa Rica

10:45 **STU** **67 A phylogenetic hypothesis on the evolution and interactions of Xenorhabdus spp. and their Steinernema hosts** Ming-Min Lee, Department of Entomology, University of Arizona, Tucson, AZ 85721, USA

11:00 **STU** **68 Plant lectins showed anti-pinewood nematode activity in vitro** Xiu Yun Zhao, College of Life Science and Technology,Huazhong Agricultural University, Shizishanjie 1, Wuhan 4

11:15 **STU** **69 Movement of adult populations of Steinernema carpocapsae searching mating partners** Yolanda Reyes, Centro de Investigación en Alimentación y Desarrollo CIAD A.C.

11:30 **STU** **70 Increased infectivity in Steinernema websteri IJ after development in desiccation stressed hosts** David Easterhoff, Marian College

11:45 **71 Parasitism of Subterranean Termites (Isoptera: Rhinotermitidae: Termitidae) by Entomopathogenic Nematodes (Rhabditida: Steinernematidae; Heterorhabditidae)** Hao Yu, Department of Entomology, University of Arizona

12:00 **72 I know what you have in your stomach: unveiling the secrets of the bacterial vesicle of Steinernema nematodes.** Sam-Kyu Kim, Univeristy of Arizona, Department of Entomology

12:15 **73 Do host plant toxins protect Drosophila larvae from wasp parasitism?** Neil Milan, Dept. of Biology, Emory University, Atlanta, Georgia, USA

Fungi Division Symposium I Tuesday 10:30 - 12:30 , Room VCH-3880
"Are entomopathogenic fungi only entomopathogens?
Conveners: Mark Goettel & Jacques Brodeur

10:30 **74 Evolution of entomopathogenicity in fungi** Richard A. Humber, USDA-ARS Plant Protection Research, US Plant Soil & Nutrition Lab., Tower Rd., Ithaca, NY 14853

10:50 **75 Entomopathogenic fungal endophytes.** Fernando E. Vega, Sustainable Perennial Crops Laboratory, USDA, ARS

11:10 **76 Beauveria bassiana: endophytic colonization and plant disease control** Bonnie Ownley, The University of Tennessee

11:30 **77 Lecanicillium spp: aphids and beyond.** Mark Goettel, Lethbridge Research Centre, Agriculture & Agri-Food Canada

11:50 **78 Entomopathogenic fungi and their relationships with the rhizosphere.** Raymond St. Leger, University of Maryland, College Park, MD, USA

12:30 - 14:00 LUNCH

12:45 DEPARTURE FOR EXCURSION (Lunch included)

18:00 Evening: BBQ and ENTERTAINMENT

WEDNESDAY, AUGUST 15TH

Contributed Papers Wednesday, 8:00 - 10:00, Room VCH-2850
Bacteria 2
Chair: Brian Federici

08:00 **79 Entomopathogenic bacteria are more virulent than mammalian pathogens to the infection model insect Galleria mellonella** Christina Nielsen-LeRoux 1Unité Génétique Microbienne et Environnement, INRA, la Minière, 78285 Guyancourt, France

08:15 **STU** **80 Characterization and role of an iron dependent internalin-like protein expressed during infection.** Nadine Daou 1Unité Génétique Microbienne et Envi ronnement, INRA, La Minière, 78285 Guyancourt cedex, France

08:30 **STU** **81 The effects of a bacterial toxin are modulated by the regiolocalization of its specific receptor at the cell surface** Onya Opota 1Institut National de la Recherche Agronomique, Centre de Sophia-Antipolis Agrobiotech, UMR 1112

08:45 **STU** **82 Single point mutations in the Manduca sexta cadherin receptor that affect binding and toxicity of Cry1A toxins** Sabino Pacheco National Autonomous University of Mexico. Biotechnology Institute (IBT-UNAM)

09:00 **STU** **83 Cell-binding and oligomerization of parasporin-2 are mediated by glycosylphosphatidylinositol -anchored proteins.** Yuichi Abe Dept. of Chem., Fac. of Sci., Kyushu University.

09:15 **STU** **84 Role of Cysteine on protein folding and biological activity of the binary toxin BinB from Bacillus sphaericus.** Patcharaporn Boonyos, Asst.Prof. Institute of Molecular Biology and Genetics, Mahidol University, Salaya

09:30 **86 Role of septicemia in the Bacillus thuringiensis mode of action.** Jorge E Ibarra Depto. de Biotecnología y Bioquímica, CINVESTAV, Irapuato, Gto., México.

Contributed Papers Wednesday, 8:00 - 10:00, Room VCH-2880
Fungi 2
Chair: Nguya Maniania

08:00 **88 Evaluation of Metarhizium anisopliae morphotypes after UV-radiation and storage** Anastasia Maljarchuk, University of Vermont, USA

08:15 **89 Hydrophobins and the spore coat of the entomopathogenic fungus Beauveria bassiana** Nemat Keyhani University of Florida

08:30 **90 Directed adaptation of entomopathogenic fungi** Nemat Keyhani University of Florida

08:45 **91 Process of infection of armoured scale insects (Diaspididae) by the entomopathogenic fungus, Fusarium** Nicola Mauchline The Horticulture and Food Research Institute of New Zealand LTD (HortResearch)

09:00 **93 Avoidance of entomopathogenic strains of Metarhizium anisophliae by termites: An evolutionary perspective** Nguya K Maniania, International Centre of Insect Physiology and Ecology (icipe), Nairob

09:15 **94 A study of gene expression of the entomopathogenic fungus Beauveria bassiana on different insect cuticles and synthetic medium through CDNA-AFLP technique** Uma Devi Andhra University

Contributed Papers Wednesday, 8:00 - 10:00, Room VCH-3860
Viruses 2 Genes and Genomes
Conveners: Eric Carstens & Nor Chejanovsky

08:00 **95 Ha44 is an essential gene for HearNPV infection and Arg25 is critical for HA44 nuclear localization** Zhihong Hu Wuhan Institute of Virology, Chinese Academy of Sciences

08:15 STU **96 The role of ME53 in Baculovirus infection** Jondavid de Jong Dept. of Molecular and Cellular Biology, University of Guelph, Guelph, ON, N1G 2W1

08:30 STU **97 Characterization of six new Mamestra configurata peritrophic matrix proteins and interaction of MacoNPV enhancin with insect intestinal mucins** Umut Toprak Agriculture and Agri-Food Canada, 107 Science Place, Saskatoon, SK, Canada S7N 0X2

08:45 STU **98 Sequence analysis of a new isolate of Cydia pomonella granulovirus (I12) that breaks CpGV resistance in codling moth** Karolin Eberle DLR Rhe inpfalz, Abteilung Phytomedizin, Biotechnologischer Pflanzenschutz, 67435 Neustadt, Deut

09:00 STU **99 ORF390 of white spot syndrome virus genome is identified as a novel anti-apoptosis gene** YI-PENG QI State Key Laboratory of VirologyCollege of Life Sciences, Wuhan University, Wuhan, P. R. China

09:15 **100 Gene Organization and content of the Western tent caterpillar, Malacosoma californicum pluviale nucleo polyhedrovirus** Shannon Escasa 1Laboratory for Molecular Ecology, Great Lakes Forestry Centre, Sault Ste. Marie, ON, P6A 2E5, 2

09:30 **101 Genotypic and phenotypic variation of South African isolates of Helicoverpa armigera single nucleocapsid nucleopolyhedrovirus** Gustav Bouwer School of Molecular and Cell Biology, University of the Witwatersrand, Johannesburg, Private Bag

09:45 **102 Structural and ultrastructural alterations of Malpighian tubules of Anticarsia gemmatalis larvae infected with different Anticarsia gemmatalis multiple nucleopolyhedrovirus (AgMNPV) recombinant viruses** Bergmann Ribeiro Departamento de Biologia Celular, Graduate Program in Molecular Biology, Universidade de Brasíli

10:00 - 10:30 COFFEE BREAK

Contributed Papers Wednesday, 10:30 - 12:30, Room VCH-2880
Microbial Control 2
Chair: Dave Chandler

10:30 **103 The Effect of Relative Humidity on the Efficacy of Different Isolates of Beauveria bassiana (Balsamo) in Microbial Control of Whitefly, Bemisia tabaci on Cucumber host Plants** Aref Olleka, Laboratory of Biological Control, College of Natural Resources and Environment, South China Agricultural University

10:45 **104 A simulation model of the greyback cane grub and its pathogens, with special emphasis on Metarhyzium anisopliae** Francis Drummond, University of Maine

11:00 **105 Preliminary results of a field trial using Beauveria brongniartii-conidiospores against the forest cockchafer, Melolontha hippocastani during the main flight in 2006** Kerstin Jung BBA, Institute for Biological Control

11:15 **106 Effects of the Clover Root Weevil Pathogen Beauveria bassiana F418 on Soil Invertebrates and Above Ground Non-target Coleoptera in New Zealand Pastures** Michael Brownbridge AgResearch, Lincoln, Christchurch 8140

11:30 **107 Fungal pathogen for biocontrol of ticks** Nguya K Maniania International Centre of Insect Physiology and Ecology (icipe), GPO Nairobi,

11:45 **108 Expressing an insect-specific scorpion neurotoxin makes Metarhizium anisopliae hypervirulent to mosquitoes, beetles and caterpillars.** Raymond St-Leger, University of Maryland

12:00 **109 Whey-based fungal micro-factories for in situ production of biological control fungi** Scott Costa Department of Plant and Soil Science, University of Vermont

12:15 **110 Impact of a treatment of Beauveria bassiana (Deuteromycota: Hyphomycetes) on honeybee (Hymenoptera: Apidae) colony health and on varroa mites (Acari: Varroidae)** William Meikle European Biological Control Laboratory, USDA-ARS

IFENSB Session III Wednesday, 10:30 - 12:30, Room VCH-2880
Infection & Stress Biology
Moderator: Bornstein Forst

10:30 **111 Entomopathogenic Nematodes from Xerophitic Habitats: Diversity, Distributional Patterns and Ecological Notes - Stress Biology and Ecology Session]** S.Patricia Stock Department of Entomology, University of Arizona, Tucson, AZ USA.

10:55 **112 Low Temperature Infection and Survival Strategies of Entomopathogenic Nematodes** Ian Brown Biology, Georgia Southwestern State University, Americus, Georgia, USA

11:20 **113 Trait modification in entomopathogenic nematodes** David Shapiro-Ilan USDA-ARS, SE Fruit and Tree Nut Research Lab

11:45 **114 Sex-specific dispersal and infection in Steinernema spp** Christine Griffin Department of Biology, NUI Maynooth, Ireland

Contributed Papers Wednesday, 10:30 - 12:30, Room VCH-3860

Viruses 3: Molecular aspects of Virus-host Interaction

Conveners: David Theilmann & Victor Romanovski

10:30 **115 Transcriptomics of the baculovirus Choristoneura fumiferanamulticapsid nucleopolyhedrovirus (CfMNPV)** Dan-Hui Yang Department of Molecular and Cellular Biology, University of Guelph, Guelph, ON, Canada

10:45 **STU** **116 Reprogramming the Autographa californica multiple nucleopolyhedrovirus chitinase expression profile** Jeffrey Hodgson University of Guelph

11:00 **STU** **117 Escape mutants of Autographa californica multiple nucleopolyhedrovirus (AcMNPV) resistant to nucleoside analogues** David Thumbi Department of Molecular and Cellular Biology, University of Guelph, ON, Canada

11:15 **STU** **118 Functional analysis of a putative inhibitor of apoptosis (IAP) encoded by Chilo iridescent virus** Ikbal Agah Ince, Karadeniz Teknik Universitesi

11:30 **119 Deletion within the AcMNPV IE0 N-terminus 54 amino acids reduces its ability to support viral DNA replication** Yingchao Nie Department of Plant Science, Faculty of Land and Food Systems, University of British Columbia

11:45 **120 The baculovirus occlusion-derived virus envelope protein P74 requires site-specific cleavage by insect midgut trypsins for function in per os infection.** Jeffrey Slack Great Lakes Forestry Centre

12:00 **121 Functional analysis of HearNPV putative anti-apoptotic genes** Changyong Liang

12:15 **122 Baculovirus infection of an insect host immuno suppressed with cys-motif and vankyrin polydnavirus genes** Nor Chejanovsky Entomology Department, Institute of Plant Protection, The Volcani Center, Bet Dagan

Student Committee Wednesday 12:00 - 14:00, Room VCH-3880

Student Committee Session with Pizza Lunch

Advisor: Patricia Stock

12:00 **123 Skills in Getting A Position in Private Industry** Ramon Georgis Valent Biosciences Corporation

12:30 **124 Academic interviews: get one step ahead** Bryony Bonning Department of Entomology, Iowa State University, Ames, IA 50011, USA

13:00 **125 The road from PhD to tenure track faculty** Juan Luis Jurat-Fuentes Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN 37996

13:30 **126 Job hunting strategies for government employment** Fernando E Vega, Sustainable Perennial Crops Laboratory, USDA, ARS

12:30 - 14:00 LUNCH (general)

12:30 - 14:00 JIP Editorial Board Meeting Room VCH-1039C

Cross-Divisional Symposium Wednesday 14:00 - 16:00, Room VCH-2850

Advances in the use of Microbial Agents for Control of Orchard Pests

Conveners: Lawrence A. Lacey & Charles Vincent

14:00 **127 Codling moth granulovirus: Learning from Europe while defining use strategies for North American orchardists** Donald Thomson DJS Consulting Services

14:15 **128 Field resistance to Cydia pomonella granulovirus - a new challenge for the biological control of codling moth** Johannes A Jehle DLR Rheinpfalz

14:30 **129 Entomopathogenic nematodes for control of codling moth in pome fruit** Lawrence Lacey Yakima Agricultural Research Laboratory, USDA-ARS

14:45 **130 Use of Bacillus thuringiensis for the control of Lepidopteran pests in apple orchards.** Jean-Charles Côté Agriculture and Agri-Food Canada

15:00 **131 Developing an IPM system with entomopathogenic nematodes for guava in Brazil** Claudia Dolinski Universidade Estadual do Norte Fluminense Darcy Ribeiro

15:15 **132 Microbial control in Florida citrus groves: problems, perils, and potential for enhancing the effectiveness of entomopathogenic nematodes for root weevil control** Robin Stuart Citrus Research and Education Center, IFAS, University of Florida, 700 Experiment Station Road,

15:30 **133 Microbial control of plum curculio and peachtree borers** David Shapiro-Ilan USDA-ARS, SE Fruit and Tree Nut Research Lab

Microsporidia Division Symposium Wednesday 14:00 - 16:00, Room VCH-2880

Microsporidia of beneficial and pest insects in greenhouse, nursery and pollination systems

Conveners: Regina Kleespies and Lee Solter

14:00 **134 A unique microsporidium infecting the black vine weevil (Coleoptera: Curculionidae) a pest of landscape, small fruit and nursery plants** Denny Bruck USDA-ARS Horticultural Crops Research Laboratory

14:25 **135 Plight of the bumblebee: Pathogen spillover from commercial to wild populations.** Sheila Colla York University, Toronto

14:45 **136 The Nosema Riddle: Puzzling fitness effects in the Bumblebee Bombus terrestris** Oliver Otti Experimental Ecology, Institute of Integrative Biology Zurich (IBZ), ETH Zurich, 8092 Zurich, Sw

15:05 **137 The convergent lady beetle and its microsporidium: potential impacts on non-target coccinellids** Taro Saito Department of Biology, Saint Mary's University, Halifax, NS CANADA

15:25 **138 Microsporidia of predaceous mites used for biological pest control** Susan Bjornson Department of Biology, Saint Mary's University, Halifax, NS CANADA

15:40 **139 Dissemination of the biocontrol agent, Vairimorpha necatrix, by the spined soldier bug, Podisus maculiventris** Rachel Down Central Science Laboratory, Defra, Sand Hutton, York, YO41 ILZ, UK

Contributed Papers Wednesday 14:00 - 16:00, Room VCH-3860

Bacteria 3

Chair: Mario Soberon

14:00 **140 Mutagenic analysis of putative domain II and surface residues in mosquitocidal Bacillus thuringiensis Cry19Aa toxin** Jong Yul Roh Department of Agricultural Biotechnology, College of Agriculture and Life Sciences, Seoul Nation

14:15 **141 Use of by-products rich in carbon and nitrogen as a nutrient source to produce Bacillus thuringiensis based biopesticide.** Fernando Valicente Embrapa

14:30 **142 Phagocytic activity and encapsulation rate of Galleria mellonella larvae hemolymph during bacterial infection Bacillus thuringiensis** Ivan Dubovskiy Institute of systematics and ecology of animals SB RAS, Russia, Novosibirsk

14:45 **STU** **143 AFM imaging of Bacillus thuringiensis Cry1 toxins interacting with insect midgut apical membranes** Éric Laflamme Université de Montréal

15:00 **144 Quantitative Cry toxin binding analyses using time resolved fluorescence** Juan Luis Jurat-Fuentes Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN 37996

15:15 **146 A rapid and highly sensitive assay for evaluating Bacillus thuringiensis strains for their insecticidal activity toward target insect pests** Algimantas Valaitis USDA Forest Service

15:30 **147 Structural changes of the Cry1Ac oligomeric pre-pore from Bacillus thuringiensisinduced by N-Acetyl galactosamine facilitates toxin membrane insertion** Liliana Pardo López Instituto de Biotecnología, Universidad Nacional Autónoma de México, Apdo. Postal

Contributed Papers Wednesday 14:00 - 16:00, Room VCH-3880

Viruses 4, Virus Production, Infection and Biotechnology

Conveners: Basil Arif & Monique Van-Oers

14:00 **148 Translation of complex baculovirus mRNAs: an unanswered question?** Ian Smith Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara, Japan

14:15 **149 Identification of retroviruses sequences in insect cells used for baculovirus expression** George Rohrmann Dept. of Microbiology Oregon State University

14:30 **150 Establishing a Tissue Culture System for the Mosquito Iridescent Virus (RMIV) from Ochlerotatus taeniorhynchus** James Becnel USDA, ARS, Gainesville, FL

14:45 **151 Production of LdNPV in the Wave® cell culture bioreactor: Comparison to production in a stirred tank bioreactor** James Slavicek, USDA Forest Service

15:00 **152 Insecticidal activity of the baculovirus expressed, basement membrane-degrading protease, ScathL** Bryony Bonning Department of Entomology, Iowa State University, Ames, IA 50011, USA

15:15 **153 Impact of a basement membrane-degrading protease on dissemination and secondary infection of Autographa californica multiple nucleopolyhedrovirus in Heliothis virescens (Fabricus)** Huarong Li Department of Entomology, Iowa State University, Ames, IA 50011, USA

15:30 **154 Infection of two lepidopteran cell lines with Amsacta moorei entomopoxvirus and induction of apoptosis** Srini Perera Great Lakes Forestry Centre, Sault Ste. Marie, ON, Canada.

15:45 **155 Isolation and characterisation of the Serratia entomophila anti-feeding prophage - a unique toxin delivery system?** Mark Hurst, Agresearch

16:00 - 16:30 COFFEE BREAK

Wednesday, 16:00 - 18:30, Pavillon Vachon, 2nd Floor

POSTER SESSION II: Fungi, Nematodes, Microsporidia, Virus II

Fungi

F-01 **Beauveria: the emergence of a new classification** Richard A Humber USDA-ARS Plant Protection Research, US Plant Soil & Nutrition Lab., Tower Rd., Ithaca, NY

F-02 **Endophytic and entomopathogenic characteristics of a fungus in the genus Colletotrichum** Jose Marcelino University of Vermont

F-03 **Surface properties of Beauveria bassiana single cell types** Nemat Keyhani University of Florida

F-04 **Genetic and phenotypic variation in Metarhizium anisopliae isolated from golf courses in Québec** Parivash Shoukouhi AAFC, Eastern Cereal and Oilseed Research Centre, Ottawa, Ontario, Canada K1A 0C6

F-05 **Morphological and molecular characterization of some new entomopathogenic fungi originating from soils in Central Brazil, and their activity against Triatoma infestans** Luiz Rocha Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, CP 131,

F-06 **Entomopathogenic fungi infecting the Asian citrus psyllid, Diaphorina citri (Homoptera: Psyllidae), in Florida** Drion Boucias University of Florida

F-07 **Surveys of indigenous entomopathogenic fungi and nematodos of Chile and studies on their pathogenicity towards pests of economic importance** Loreto Merino Instituto de investigaciones agropecuarias Chile

F-08 **Occurrence of invertebrate-pathogenic fungi in a Cerrado ecosystem in Central Brazil** Luiz Rocha Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, CP 131, 74001-97

F-09 **Detection of chalkbrood of honeybees (Apis mellifera) caused by the fungus Ascosphaera apis** Sung Hee Nam National Institute of Agricultural Science and Technology, R. D. A. Suwon, Korea

F-10 **Annotated checklist of arthropod-pathogenic fungi from Brazil and Argentina** Daniel Sosa-Gomez Embrapa Soybean, Cx.P. 231 Londrina, PR 86001-970, Brazil

F-11 **Difference in aphid and fly host driven divergence of Entomophthora species** Jørgen Eilenberg University of Copenhagen, Faculty of Life Sciences, Department of Ecology, Thorvaldsensvej 40

F-12 **Can Metarhizium anisopliae, really colonize the plant rhizoplane?** Stefan Jaronski USDA ARS

F-13 **Inhibition of phagocytic activity and nodulation in Galleria mellonella by the entomopathogenic fungus, Nomuraea rileyi** Tseng Yu Kai Department of Entomology, National Chung Hsing University, Taichung, Taiwan, R.O.C

F-14 STU **Pathogenicity and its mode of action of Verticillium lecanii (Lecanicillium spp.) hybrid strains against different sedentary stages of Heterodera glycines** Ryoji Shinya Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto, Japan

F-15 **Pathogenicity of Verticillium lecanii (Lecanicillium spp.) hybrid strains to different developmental stages of greenhouse whitefly, Trialeurodes vaporariorum** Sayaka Horie Department of Agro-enviromental Science, Obihiro University of Agriculture and Veterinary Medici

F-16 **Susceptibility of immature stages of the greenhouse whitefly parasitoid, Encarsia formosa, to the entomopathogenic fungus Verticillium lecanii** Ahmad Ashouri Professor

F-17 **Stresses improve Beauveria bassiana efficacy for Tribolium castaneum** Jeff Lord USDA-ARS

F-18 **Utility of fungicides to isolate invertebrate-pathogenic fungi** Luiz Rocha Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, CP 131, 74001-97

F-19 **Roll-method for mass-production of hyphomycetous fungi** Vladimir Gouli University of Vermont

F-20 **Efficiency of three different mass-production methods for hyphomycetous fungi** Svetlana Gouli University of Vermont

F-21 STU **Importance of viability of Verticillium lecanii (Lecanicillium spp.) on the cucumber leaf surface.** Daigo Aiuchi Department of Agro-environmental Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080-8555, Hokkaido, Japan

F-22 **Brief encounters or lasting relationship? Detecting and quantifying field persistence of introduced Beauveria bassiana GHA for emerald ash borer control by use of real-time PCR** Louela Castrillo Department of Entomology, Cornell University, Ithaca, NY 14853

F-23 **Selection of isolates of different Beauveria spp. for high tolerance to UV-B radiation** Donald Roberts Utah State University

F-24 **Screening, identification and determination of functional parameters of photosensitizers with antifungal action** Gilberto Braga Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto

F-25 **Visible light during growth increases tolerance of Metarhizium anisopliae var. anisopliae conidia to UV-B radiation, but does not alter heat tolerance nor conidial yield** Donald Roberts Utah State University

F-26 **Harvesting of insecticidal chitinase produced from entomopathogenic fungi, Beauveria bassiana DBB2507 using Enzyme Absorption Method** Jae Su Kim AgroLife Research Institute, Dongbu Hannong Co. Ltd., South Korea

F-27 **Induction of apoptosis in Sf-21 cell line by cultured fluid of the entomopathogenic fungus, Nomuraea rileyi** Roger F Hou Department of Entomology, National Chung Hsing University, Taichung 402, Taiwan, ROC

F-28 **Tyrosine betaine: a new biomolecule isolated from Metarhizium anisopliae conidia** Gilberto Braga Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto

F-29 **Genotypic variability among Brazilian isolates of Beauveria bassiana** Everton Fernandes Utah State University

F-30 **Identification of Metarhizium anisopliae transcripts expressed during the fungus- insect interaction.** Bruno Donzelli USDA - Agricultural Research Service

Nematodes

N-01 **The host-parasite biology of Frankliniella fusca (Hinds) (Thysanoptera: Thripidae) and Thripinema fuscum Tipping & Nguyen (Tylenchida: Allantonematidae)** Kelly Sims University of Florida

N-02 **Stable association between Serratia marcescens and Steinernema carpocapsae during the infection process.** Jorge E Ibarra Depto. de Biotecnología y Bioquímica, CINVESTAV, Irapuato, Gto., México

N-03 **To protect and enhance conservation and sustainable use of the entomopathogenic nematode biodiversity of Chile** DAVE MOORE CABI UK, Silwood Park, Buckhurst Road, Ascot, Berks SL5 7TA, UK.

N-04 **Diversity of entomopathogenic Nematodes (Steinernematidae, Heterorhabditidae) in Jordan** S.Patricia Stock Department of Entomology, University of Arizona, Tucson, AZ 85721, USA

N-05 STU **N-acetyl alpha-D-glucosaminidase, a cuticle enzyme secreted by axenic Steinernema carpocapsae suppresses the immune system of the Greater wax moth, Galleria mellonella** Jason Lapointe McGill University, MacDonald Campus; Anatomy and Cell Biology Graduate Program

N-06 STU **Blackfly mermithids from Québec : an ecological and molecular study** Mylène St-Onge Département Chimie-Biologie, Université du Québec à Trois-Rivières, Québec, Canada

N-07 **Detection and sequence analysis of insecticidal gene tccC1/xptB1 homologues from Xenorhabdus nematophilus (CR5), bacterial symbiont of the entomophagous nematode Steinernema westerii (Rhabditida: Steinernematidae) isolated from northern Costa R** Marielos Mora University of Costa Rica

N-08 STU **Investigation on biological control of alfalfa stem nematode (Ditylenchus dipsaci) by using Rhizoglyphus robini** Omid Joharchi

N-09 **Managing chickpea pod borer, Helicoverpa armigera (Hub.) with Heterorhabditis indica A success story** Aralimarad Prabhuraj, Department of Entomology, College of Agriculture, Raichur 584 101, Karnataka, India

N-10 **Metabolites from axenic Steinernema carpocapsae suppress the non-self responses of the pest insect Galleria mellonella** Ndonkeu Tita Walter McGill University

N-11 **Some anti-nematodes compounds as candidates for a trunk-injection agent against the pine wilt disease** DongWoon Lee Sangju National University

N-12 **Biological control of root knot nematode, Meloidogyne hapla using plant extracts** DongWoon Lee Sangju National University

N-13 **Biocontrol of Meloidogyne incognita juveniles by Plant Extracts** DongWoon Lee Sangju National University

Microsporidia

MS-02 **The influence of seasonality and red imported fire ant (Solenopsis invicta) caste and colony social form on the prevalence and spore titer of the microsporidium Thelohania solenopsae.** Maynard Milks Department of Entomology, Louisiana Agricultural Experiment Station, Louisiana State Universit

MS-03 **Effects of a novel microsporidian isolate from Poland on larvae of the Gypsy Moth (Lymantria dispar L.)** Andreas Linde University of Applied Sciences at Eberswalde, Faculty of Forestry, Alfred-Moeller-Str. 1

MS-05 **A new Vairimorpha isolate from Ocinara lida in Taiwan** Yi-Chun Tsai Department of Entomology, National Taiwan University, Taipei 106, Taiwan

MS-06 **Molecular data and phylogeny of Nosema infecting Lepidopteran forest defoliators in the genus Choristoneura and Malacosoma** George Kyei-Poku Natural Resources Canada, Canadian Forestry Service, Great Lakes Forestry Centre, 1219 Queen Str

Viruses II

V-26 **Development of a direct cloning system for the baculovirus, Anticarsia gemmatalis Multiple Nucleopolyhedrovirus (AgMNPV).** Jeffrey Slack Great Lakes Forestry Centre

V-27 **A two color tag system to study virus-virus interaction in vitro** Xiao-Wen Cheng Department of Microbiology, Miami University, Oxford, Ohio 45056 USA

V-28 **To study the insecticidal efficacy of Spodoptera exigua multiple nucleopolyhedrovirus combined with wheat germ agglutinin or concanavalin A** Tzyy-Rong Jinn Biopesticides Division, Taiwan Agricultural Chemicals and Toxic Substances Research Institute

V-29 **Molecular techniques for the detection, differentiation and quantitation of baculovirus isolates in Choristoneura fumiferana** David Woodward University of Guelph / Great Lakes Forestry Centre

V-30 **The use of biolistics to infect, transfect, and co-transfect baculoviruses in larvae.** Cristina Del Rincón-Castro Depto. de Biotecnología y Bioquímica, CINVESTAV, Irapuato, Gto., México.

V-31 **Post-transcriptional processing of baculovirus late mRNAs** Yi Li Texas A&M University

V-32 **Identification and functional analysis of Leucania separata multiple nuclear polyhedrosis virus anti-apoptosis genes** Songya Lu State Key Laboratory of VirologyCollege of Life Sciences, Wuhan University, Wuhan, P. R. China

V-33 **Functional analysis of Helicoverpa armigera nucleopolyhedrovirus ORF2** Changyong Liang, State Key Lab of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071

V-34 **ac18 is not essential for propagation of Autographa californica multiple nucleopolyhedrovirus** Kai Yang State Key Laboratory of Biocontrol, Sun Yat-sen University, Guangzhou 510275, China

V-35 **Mutagenesis and functional analysis of the fusion peptide of HearNPV F protein** Zhihong Hu Wuhan Institute of Virology, Chinese Academy of Sciences

V-36 **Identifying the key amino acids required for nuclear localization of AcMNPV late expression factor 3 (LEF-3)** Victoria Au Department of Microbiology and Immunology, Queen's University

V-37 STU **Sequence analysis of the Spodoptera litura granulovirus genome** Yong Wang Department of Agricultural Biotechnology, Seoul National University, Seoul, Korea

V-38 **Complete sequence and organization of Antheraea pernyi nucleopolyhedrovirus, a dr-rich baculovirus** Zuoming Nie Institute of Biochemistry, Zhejiang Sci-Tech University, Xiasha High-Tech Zone No.2 Road, Hangzh

V-39 **Comparative study of the sequence of Choristoneura biennis entomopoxvirus** Zhen Li Laboratory for Molecular Virology, Great Lakes Forestry Centre, Sault Ste. Marie, Ontario, P6A 2

V-40 **Role of the baculovirus P143/LEF-3 complex in viral DNA replication** Mei Yu Department of Microbiology and Immunology, Queen's University

V-41 **The 5' untranslated region of Perina nuda virus (PnV) possesses a strong internal translation activity in baculovirus infected insect cells** Tzong-Yuan Wu Department of Bioscience Technology and Center for Nanotechnology, Chung Yuan Christian Universi

V-42 STU **Restricted gene transcription of Autographa californica multiple nucleopolyhedrovirus (AcMNPV) in mammalian cells** Ryusuke Fujita Laboratory of Applied Molecular Entomology, Graduate School of Agriculture, Hokkaido University, Sapporo, Japan

V-43 **Determination of the promoter region of the Chilo iridescent virus DNA polymerase gene** Zihni Demirbag Karadeniz Technical University, Trabzon, Turkey

V-44 **Annotation and expression profiling of presumptive apoptosis regulatory genes in the yellow fever mosquito, Aedes aegypti** Rollie Clem Kansas State University

V-45 **A strategy for genetic modification of Epinotia aporema Granulovirus** Victor Romanowski IBBM, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Argentina

V-46 **Evidence supporting the presence of viral fibroblast growth factor on the surface of baculovirus virions** Chris Lehiy Molecular, Cellular, and Developmental Biology Program, Division of Biology, Kansas State Univer

V-47 **Identification of virion proteins of Spodoptera frigiperda ascovirusby mass spectroscopy** Dennis Bideshi Department of Entomology, University of California, Riverside, Riverside, CA 92521

V-48 **Sf29 is a viral factor that could be involved in virion packing within the OBs** Oihane Simon Laboratorio de Entomología Agrícola y Patología de insectos, Departamento de Producción Agraria,

V-49 **Functional and phylogenetic comparisons of viral homologues of a protein lethal to endoparasitoids** Madoka Nakai Tokyo University of Agriculture and Technology, Fuchu-shi, Japan

V-50 **Determination of the occlusion-derived virus proteins of Xestia c-nigrum granulovirus** Chie Goto National Agricultural Research Center

Wednesday 20:00 - 21:00, Room VCH-2850
Microbial Control Division Business Meeting

Workshop Wednesday 21:00 - 22:00, Room VCH-2850
Joint Workshop Organized by Microbal Control and Bacterial Divisions: To Bt or not to Bt: do we need an international reference standard? Pros, cons and future directions.
Convener: Larry Gringorten Moderator: M. Brownbridge

Specifications and Prospects of HD-1-S-2005. D. Ave
Distribution, J. Siegel
Issues, G. Benzon
Summary, W. Gelernter

THURSDAY, AUGUST 16TH

Contributed Papers Thursday 08:00 - 10:00, Room VCH-2850
Fungi 3
Chair: Denny Bruck

08:00 **156** **Evaluation of whey as a basal media ingredient for mass production of Beauveria bassiana and Metarhizium anisopliae** Adane Kassa University of Vermont, Entomology Research Laboratory, 661 Spear St. Burlington, VT 05405-0105,

08:15 **157** **Effect of culture medium on Paecilomyces fumosoroseus morphogenesis, growth and infective propagules production and properties** Ali Asaff Centro de Investigación en Alimentación y Desarrollo CIAD A.C.

08:30 **158** **Further research on the production, longevity and infectivity of the zoosporesof Leptolegnia chapmanii Seymour (Oomycota: Peronosporomycetes)** Lastra, Claudia López CEPAVE Centro de estudios parasitologicos y de vectores

08:45 **159** **Development of a formulation of Beauveria on non woven fabric strips for control of Monochamus alternatus (Coleoptera: Cerambycidae)** Mitsuaki Shimazu Forestry and Forest Products Research Institute

09:00 **160** **Investigating the structure of natural populations of Beauveria bassiana occurring in different habitats** Dave Chandler Warwick HRI, University of Warwick, Wellesbourne, Warwick CV35 9EF UK.

09:15 **161** **Association of Beauveria spp. with Bark Beetle Populations in Pinus radiata Plantations in New Zealand** Michael Brownbridge 1AgResearch, Science Centre, Gerald Street, Lincoln, Christchurch, New Zealand

09:30 **162** **Molecular characterization and comparative virulence of Beauveria bassiana isolates associated with the shore fly, Scatella tenuicosta, in greenhouses** Todd Ugine Department of Entomology, Cornell University, Ithaca, NY 14853

09:45 **163** **Horizontal transmission possibility of the fungus Beauveria bassiana KCF102 by mating behavior between Sunn pest, Eurygaster integriceps (Hem., Scutelleridae) adults** Reza Talaei-Hassanloui Department of Plant Protection, University College of Agriculture and Natural Resources, Univers

Cross-Divisional Symposium Thursday 08:00 - 10:00, Room VCH-2880
Battling alien invaders: Development and use of entomopathogens to control invasive insect pests
Convener: Louela Castrillo

08:00 **164** **Development and production of the Lymantria dispar nucleopolyhedrovirus as a microbial control agent for the gypsy moth** James Slavicek USDA Forest Service

08:20 **165** **Releasing exotic microsporidia into North American gypsy moth populations: regulations and other issues** Leellen Solter Illinois Natural History Survey

08:40 **166** **Entomophaga maimaiga and the Gypsy Moth in North America: Toward Predicting Epizootics** Ann Hajek Department of Entomology, Cornell University

09:00 **167** **Potential uses of Beauveria bassiana GHA for management of the emerald ash borer, Agrilus planipennis Fairmaire (Coleoptera: Buprestidae)** Leah Bauer USDA Forest Service, Northern Research Station, E. Lansing, MI

09:20 **168** **Thelohania solenopsae as a factor of fire ant populations** David Oi USDA-ARS, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, Florida USA

09:40 **169** **Tritrophic interaction in the control of Sirex woodwasp by nematodes** Ray Akhurst CSIRO Entomology

Contributed Papers Thursday 08:00 - 10:00, Room VCH-3860
Microbial Control 3
Chair: TBD

08:00 **170** **Use and formulation of Baculovirus insecticides in Australian broadacre crops.** Caroline Hauxwell Department of Primary Industries & Fisheries, Queensland, Australia

08:15 **STU** **170,1** **Suppressing plum curculio (Coleoptera : Curculionidae) with biopesticides** Renee Pereault Michigan State University Department of Entomology

08:30 **170,2 Identification of the midgut receptor for Cry4Ba toxin in Anopheles albimanus larvae.** T. Fernández-Luna, Departamento de Microbiología Molecular, Instituto de Biotecnología, Universidad Nacional Autónoma de México

08:45 **172 Bioactivities of Photorhabdus luminescens subsp. akhurstii, a symbiont of entomopathogenic nematode, Heterorhabditis brevicaudis** Suey-Sheng Kao Biopesticides Division, Taiwan Agricultural Chemicals and Toxic Substances Research Institute

09:00 **173 Novel Controlled-Delivery Formulation Technology: Mosquito Biolarvicide Applications** Richard Levy Lee County Mosquito Control District, Technology Development Center

09:15 **174 Quantifying the serine protease enzymes of neat gut juice from C. fumiferana (spruce budworm).** Ross Milne Great Lakes Forestry Centre, Sault Ste. Marie ON

09:30 **175 Bioassay of a highly purified vip 3a toxin against forest pest lepidoptera** Ross Milne Canadian Forestry Service Sault Ste. Marie

09:45 **176 Authorisation and commercialisation of microbial biopesticides: regulatory innovation and the regulatory state** Dave Chandler Warwick HRI, University of Warwick, Wellesbourne CV35 9EF UK

Contributed Papers Thursday 08:00 - 10:00, Room VCH-3880
Bacteria 4
Chair: Hyun-Woo Park

08:00 **177 Identification of the receptor-binding motif of the binary toxin from Bacillus sphaericus** Kamonnut Singkhamanan Institute of Molecular Biology and Genetics, Mahidol University, Salaya Campus, Nakorn pathom 731

08:15 **178 Molecular tools for detection and monitoring of an allele conferring Bacillus sphaericus resistance in Culex quinquefasciatus** Maria Helena Neves Silva-Filha, Department of Entomology, Centro de Pesquisas Aggeu Magalhães-Fundação Oswaldo Cruz

08:30 **179 Recombinant bacteria delay the evolution of resistance in mosquito larvae** Margaret Wirth Department of Entomology, University of California, Riverside, CA

08:45 **180 Interactions between ORF157, ORF156 and the iteron in replication of pBtoxis of Bacillus thuringiensis subsp. israelensis** Mujin Tang 1Department of Entomology, University of California, Riverside, California

09:00 **181 Economical overproduction of bioinsecticides of Bacillus thuringiensis by random mutagenesis, heat and salt stress, control of oxydative metabolism and adequation of fermentation technology** Nabil Zouari Center of Biotechnology of Safx, Tunisia

09:15 **182 Changes in the transcriptional profile of Heli coverpa armigera after feeding with Cry1Ac or Cry2Ab Bacillus thuringiensis toxins** Salvador Herrero Department of Genetics. University of Valencia, Spain

09:30 **183 Effects of amino acid substitutions in a highly con served region of a cytolytic toxin from Bacillus thuringiensis** Boonhiang Promdonkoy BIOTEC Central Research Unit, National Center for Genetic Engineering and Biotechnology

09:45 **184 Effects of mutagenic residues at N- and C-termini on structure and function of a cytolytic toxin from Bacillus thuringiensis** Siriya Thammachat Institute of Molecular Biology and Genetics

10:00 - 10:30 COFFEE BREAK

Thursday 10:30 - 12:30, Room PLT-1112
Business Meeting of SIP

Thursday 12:30 - 14:00, Room VCH-1039C
Student Awards Committee Luncheon Meeting

12:30 - 14:00 LUNCH

Fungi Division Symposium Thursday 14:00 - 16:00, Room VCH-2850
Fungal Secondary Metabolites: Knowns and Unknowns
Conveners: John Vandenberg, Alice Churchill & Donna Gibson

14:00 **185 Terrequinone A biosynthesis - implications beyond Aspergilli** Dirk Hoffmeister University of Minnesota-Twin Cities Campus, Plant Pathology

14:20 **186 Integration of polyketides into the life cycle of Fusarium graminearum.** Frances Trail Michigan State University

14:40 **187 Using molecular genetics to reveal metabolic pathways of Metarhizium anisopliae** Alice C.L Churchill Cornell University

15:00 **188 New secondary metabolites from Metarhizium anisopliae** Stuart B Krasnoff USDA-ARS-Plant Protection Research Unit, Ithaca, NY 14853

15:20 **189 Risk assessment of metabolites produced by entomopathogenic fungi - a REBECA statement** Hermann Strasser, Institute of Microbiology University of Innsbruck

Contributed Papers Thursday 14:00 - 16:00, Room VCH-2880
Microbial Control 4
Chair: Terry Benson

14:00 **190 The susceptibility of Anopheles mosquitoes to Bacillus thuringiensis subsp. israelensis** Gustav Bouwer School of Molecular and Cell Biology, University of the Witwatersrand, Johannesburg

14:15 **191 Species identification and host range testing of a new entomopathogenic member of the Enterobacteriaceae** Mark Hurst AgResearch, NZ

14:30 **192 A novel Bacillus thuringiensis isolate that produces cuboidal crystals and is highly toxic to larvae of Trichoplusia ni** Izabela Swiecicka Department of Microbiology, University of Bialystok, Bialystok, Poland

14:45 **193 Quasi-innate-immunity: hemolymph peptide induction with Bacillus thuringiensis (Bt) exposure and bacterial challenge in Bt-resistant and susceptible cabbage loopers, Trichoplusia ni (Hubner).** Jerry Ericsson Department of Biological Sciences, Simon Fraser University, Burnaby, BC, Canada

15:00 **193,1 cDNA microarray analysis of genes involved in parasitization of the silkworm Bombyx mori by tachinid parasitoids** Andrew Kalyebi National Institute of Agrobiological Sciences, Tsukuba, Japan

15:15 **195 Seasonal migration, local movement and the pattern of Bt resistance in cabbage looper populations in British Columbia** Judith Myers Dept. Zoology, University of British Columbia, Vancouver

15:30 **196 Response of Heliothis virescens to different diets containing the same amounts of Bacillus thuringiensis Cry1Ac** Carlos Blanco USDA Agricultural Research Service

15:45 **197 Verticillium lecanii (Lecanicillium spp.) as plant bodyguards** Masanori Koike, Department of Agroenvironmental Science, Obihiro University

Contributed Papers Thursday 14:00 - 16:00, Room VCH-3860
Viruses 5: Insect Virus Diversity and Evolution
Conveners: Peter Krell & George Rohrmann

14:00 **198 The genes driving baculovirus genome evolution** Elisabeth Herniou Division of Biology, Imperial College London, Silwood Park, Ascot, UK

14:15 **199 Trichoplusia ni and Chrysodeixis chalcites single nucleopolyhedroviruses: Genomic and biological comparison.** Martin Erlandson AAFC, Saskatoon Research Centre, 107 Science Place, Saskatoon, SK, Canada

14:30 **200 Towards the complete genome sequence of the baculovirus-related nonoccluded Oryctes rhinoceros nudivirus of beetles** Yongjie Wang Laboratory for Biotechnological Crop Protection, Department of Phytopathology

14:45 **201 Origin of Ichnoviruses : is there consistent molecular support to the Brian Federicis endosymbiogenic theory?** Yves Bigot Laboratoire dEtude des parasites Génétiques, FRE CNRS2969,

15:00 **202 Genome analysis of salivary gland hypertrophy virus (SGHV) reveals a novel large double-stranded circular DNA virus from Glossina pallidipes** Adly Abd-Alla Entomology Unit, FAO/IAEA Agriculture and Biotechnology Laboratory, Agencys Laboratories Seiber

15:15 **203 Characterization of the Musca domestica salivary gland hyperplasia virus (MdSGHV)** Drion Boucias University of Florida

15:30 **204 A caspase-like gene from Heliothis virescens ascovirus (HvAV-3e) is not involved in apoptosis but is essential for virus replication** Sassan Asgari School of Integrative Biology, University of Queensland, St. Lucia QLD 4072, Australia

15:45 **205 Two Microplitis demolitor Bracovirus virulence factors, PTP-H2 and Glc1.8, induce apoptosis in insect hemocytes** Richard Suderman University of Georgia, Department of Entomology

16:00 - 16:30 COFFEE BREAK

Workshop Thursday 16:15 - 19:30
Virus Division Satellite Workshop: The Biology of Polydnaviruses: some unresolved issues. Please note location: Laurentian Forestry Centre, Lionel-Daviault Seminar Room (Laval University Campus)

16:15 **205,1 The biology of polydnaviruses: some unresolved issues.** Don Stoltz

16:30 **205,2 Challenges in defining the funcional roles of related virulence gene variants in polydnaviruses.** Michael Strand

16:45 **205,3 Analysis of a Glyptapanteles indiensis BV proviral locus.** Dawn Gundersen-Rindal

10 MINUTE BREAK

17:00 **205,4 The sequencing of the integrated form of CcBV: one locus or several loci?** Jean-Michel Drezen

17:15 **205,5 Genomic organisation of the ichnovirus HdIV.** Nathalie Volkoff

17:30 **205,6 Polydnaviruses and virus-like particles as wasp extended genotypes and phenotypes.** Sassan Asgari

Round-Table Discussions

IFENSB Session IV Thursday 16:15 - 19:30, Room VCH-2850
ECOLOGY
Moderator: Claudia Dolinski

16:15 **206 Entomopathogenic nematodes in heterogeneous soils: foraging behavior and infection decisions.** Glen Stevens Department of Nematology, Univ. of CA, Davis

16:35 **207 Host behavioral response** Albrecht Koppenhöfer Dept. Entomology, Rutgers University

17:55 **208 Soil food webs, entomopathogenic nematodes, and biological control: shedding some light on an old black box** Robin Stuart University of Florida, IFAS, Citrus Research and Education Center, Lake Alfred, FL, 33850 USA

18:15 **209 Modeling entomopathogenic nematode population patterns and processes in ecosystems** Casey Hoy The Ohio State University, Ohio Agricultural Research and Development Center

Contributed Papers Thursday 16:15 - 18:15, Room VCH-3860
Bacteria 5
Chair: Christina Nielsen-LeRoux

16:15 **210 Adult non-biting midges: possible windborne carriers of Vibrio cholerae** Meir Broza Faculty of Science and Science Education, University of Haifa, Oranim, Tiv'on, 36006, Israel

16:30 **211 Isolation of an insect-active super toxin complex from a new species of the Yersinia** Mark Hurst

16:45 **212 Exploring the use of RNAi for insect control** James Baum Monsanto Company

17:00 **213 Symbiotic bacteria, heat stress and pea aphid resistance to parasitoids** Jean-Frédéric Guay Département de Biologie, Université Laval

17:15 **214 Isolation, and Characterization of Two Toxin Complexes from Xenorhabdus nematophilus** Joel Sheets Dow AgroSciences

17:30 **215 Chemical mutagenesis of Heliothis virescens causes resistance to multiple Bacillus thuringiensis proteins** Omaththage Perera Southern Insect Management Research Unit, USDA-ARS

17:45 **216 Establishment of a proteomic approach to study brush border membrane proteins in Lepidopteran larvae** Yannick Pauchet Max Planck Institute for Chemical Ecology, Entomology department

18:00 **217 Bacillus thuringiensis: A very attractive bacterium for various biotechnological applications.** Samir JAOUA Center of Biotechnology of Sfax. Laboratory of Biopesticides. P.O.Box. K. 3038. Sfax. Tunisia

Contributed Papers Thursday 16:15 - 19:30, Room VCH-3880

Microbial Control 5

Chair: Judith Pell

16:15 **218 Comparative virulence of three hyphomycetous fungi against the bollworm, Helicoverpa armigera, employing topical versus per os inoculation techniques** Justin L Hatting ARC-Small Grain Institute, Private Bag X29, Bethlehem, 9700, South Africa

16:30 **219 Integration of soil inoculation with Metarhizium anisopliae into bait-based technology for field suppression of Bactrocera invadens on mango** Sunday Ekesi International Centre of Insect Physiology and Ecology (icipe), PO Box 30772-00100 GPO, Nairobi,

16:45 **220 Linkage and mapping analysis of a gene resistant to Bacillus thuringiensis Cry1Ab toxin in the silkworm Bombyx mori** Shogo Atsumi

17:00 **221 Integrated applications of Bacillus thuringiensis serovar. tenebrionis and Beauveria bassiana for biologi cally-based integrated pest management of Colorado potato beetle** Stephen Wraight USDA-ARS-PPRU, U.S. Plant, Soil, and Nutrition Laboratory, Ithaca, NY USA

17:15 **223 Efficacy trials of Beauveria bassiana and Metarhizium anisopliae for Pieris rapae (Lepidoptera: Pieridae) control on commercial cabbage cultures** Cipriano García Gutiérrez

17:30 **224 Biology of Sunn Pest (Eurygaster integriceps) (Hemiptera: Scutelleridae) relevant to control with a mycoinsecticide** Dave Moore CABI

17:45 **225 Beauveria bassiana plus chemical attractant: A new approach against pine sawyer?** Zengzhi Li Department of Forestry, Anhui Agricultural University, Hefei, Anhui230036, P.R.China

19:00 - 02:00 BANQUET

19:00 – 02:00 **Banquet & Award Ceremony at the Delta Hotel**

19:00 – 19:45 Shuttles to the Banquet:

18:45 A bus will leave **Classique Hotel** to the Banquet

18:50 A bus will leave **Universel Hotel** to the Banquet

19:00 **Buses will leave Pavillon Moraud to the Banquet**

19:20 A bus will leave **Universel Hotel** to the Banquet

19:30 **Buses will leave Pavillon Moraud to the Banquet**

19:45 A bus will leave **Pavillon de la Foresterie** to the Banquet

19:00 – 20:00 **Cocktail Hour**

20:00 **Banquet**

22:30 – 02:00 **Buses back to the University and Hotels**

ABSTRACTS
2007

MONDAY, AUGUST 13TH

PLENARY LECTURES. Monday 10:30 - 12:30

Symposium. Monday, 10:30. (1)

Looking back: 40 years of SIP

Elizabeth W. Davidson
School of Life Sciences, Arizona State University, Tempe, AZ 85287-4501 USA

Our meeting in Quebec City will be the 40th meeting of SIP. It is time to look back at the colleagues who were critical to the development of our field. We will highlight several of the laboratories worldwide that had a major impact on invertebrate pathology, but sadly, are no longer active in the field. Many of us received training in those laboratories, or base our current research on groundbreaking discoveries made there. The Society has benefited greatly from the efforts of these laboratories in hosting meetings, and in the service of their members as officers. It is time to remember and honor them.

Symposium. Monday, 11:30. (2)

Chemical ecology and invertebrate pathology: Do sub-lethal pathogenic infections affect chemically mediated behaviors?

Jeremy N. McNeil 1 and Jacques Brodeur 2
1 Department of Biology, University of Western Ontario, London, ON, Canada
2 Département de Sciences biologiques, Université de Montréal, Montréal, QC, Canada

Considerable attention has been given to the sub-lethal effects of pathogenic infections on life history parameters of invertebrates, such as developmental time, body mass, fecundity and adult longevity. However, the possibility that infected individuals may have reduced abilities to either produce or detect chemical cues of importance in the location of food sources, oviposition sites and mates, as well as the detection of natural enemies has not been examined in great detail.

In this presentation we will look systems that might be affected, for we believe that while the effects may be subtle, they could have a significant impact on the population dynamics of infected hosts and could provide interesting avenues of future collaborative research work.

SYMPOSIUM, Bacterial Division, Monday 14:00 - 16:00

Mode Of Action Of Bacillus Thuringensis Cry Toxins

Symposium. Monday, 14:00. (3)

New Insight into the Mode of Action of Bacillus thuringiensis Cry Toxin: Cell Death by cAMP

X. Zhang, N. B. Griko and L. A. Bulla, Jr.
Biological Targets, Inc., Pilot Point, TX and The University of Texas at Dallas, Richardson, TX

Cell death in insects susceptible to Cry toxin is initiated by the binding of toxin to a cadherin receptor. Toxin binding, localized on the EC12 module of the receptor, is strictly univalent and occurs with no oligimerization of either the toxin or the receptor. Binding of Cry toxin to receptor on the cell surface evokes an intracellular signal that stimulates G protein and adenyl cyclase (AC) activity, along with a dramatic increase in production of cAMP. cAMP activitates protein kinase A (PKA), bringing about an array of cellular changes including cytoskeletal rearrangement and ion fluxing. Toxin-induced cell signaling also involves exocytotic trafficking of the receptor to the cell surface, which, in turn, recruits additional toxin. Receptor enrichment of the cell surface amplifies the signal cascade initialized by toxin-receptor interaction. Cry toxin action is a dynamic process that involves binding of toxin to receptor, stimulation of heterotrimeric G protein and AC and creation of the second messenger cAMP. Acceleration of this second messenger pathway alters the chemistry of the cell to elicit cell death by oncosis.

Symposium. Monday, 14:30. (4)

The pre-pore oligomer is an obligate intermediate in the cell death induced by Bacillus thuringiensis Cry1Ab toxin in insect larvae.

Nuria Jimenez-Juárez, Isabel Gómez, Ivan Arenas, Liliana Pardo, Carlos Muñoz-Garay, Sarjeet S. Gilla, Alejandra Bravo and Mario Soberón.
Instituto de Biotecnología, Universidad Nacional Autónoma de México. Apdo. postal 510-3, Cuernavaca 62250, Morelos, Mexico, and aDepartment of Cell Biology and Neuroscience, University of California, Riverside, CA 92506.

Cry toxins of Bt are pore forming toxins, their primary action is to lyse midgut epithelial cells in target insect. In Manduca sexta, a cadherin-like protein (Bt-R1) and aminopeptidase-N (APN), were described as Cry1A-receptors. Previously, we showed that binding of monomeric Cry1Ab toxin to Bt-R1 promotes the formation of a pre-pore oligomeric structure that is competent in membrane insertion. The oligomeric Cry1A structure then binds to APN receptor leading to its insertion into membrane lipid rafts implying a sequential binding mechanism of Cry1A toxins with Bt-R1 and APN. In this work we will present two sets of data that support the pore-forming model. We identified antibody (scFvM22) that recognizes β16-β22 in domain III. ELISA and toxin overlay binding competition assays in the presence scFvM22 showed that domain III β16 is involved in the interaction of the pre-pore oligomer with APN. scFvM22 lowered the toxicity of Cry1Ab to M. sexta larvae indicating that interaction with APN is important for in vivo toxicity. Additionally, we show that helix α-3 from domain I contains sequences that could form coiled-coil structures important for oligomerization. Single point mutations in helix α-3 resulted in proteins that bind Bt-R1 with a similar KD as the Cry1Ab toxin but unable to form oligomeric structures in vitro. These mutants were also severely affected in pore formation and toxicity, indicating that the pre-pore oligomer is an obligate intermediate in the intoxication process of Cry1Ab toxin in insect larvae.

Symposium. Monday, 15:00. (5)

A Critique of Current Models for the Mode of Action of BT Toxins

Donald H. Dean
Department of Biochemistry, The Ohio State University, Columbus, Ohio 43210, USA.

Three models for the mode of action of the Cry toxins of Bacillus thuringiensis will be considered. The Umbrella Model, the Serial-Receptor-Binding Model and the Signal Transduction Model will be describe. Data will be presented that all three domains of Cry toxins are sequestered into the membrane. Further, a specific mutation, F371A, in domain II blocks insertion of the toxin into the membrane. This mutant binds normally to purified cadherin and competitively, as wild-type, to BBMVs. These data are in disagreement with the Umbrella and Signal Transduction Models. Further data will be presented that a specific mutation in domain III of Cry1Aa, blocks binding to cadherin but retains most of its toxicity. This observation, and the published observation of a mutation in domain III of Cry1Ac that blocks binding to APN but retains most of its toxicity, indicate that Cry toxins may function by binding to more than one receptor, any one of which may lead to toxicity. This conclusion is in disagreement with the Serial-Receptor-Binding Model. The simplest model consistent with all of the data in one in which toxins may bind in parallel to several receptors and insert, via all three

domains, into the membrane forming a pore.

Symposium. Monday, 15:30. (6)

Thinking outside the box for the design of Bt products: can we take advantage of their target cells' environment and physiological response to aggression?

Jean-Louis Schwartz1,2, Vincent Vachon1,3 and Raynald Laprade1,3

1Groupe d'étude des protéines membranaires (GÉPROM-FRSQ), 2Department of Physiologie and 3Department of Physics, Université de Montréal, P.O. Box 6128, Centre-Ville Station, Montréal, Québec, Canada H3C 3J7

Following recognition of specific binding sites at the surface of midgut target cell membranes, activated Bacillus thuringiensis (Bt) toxins act mainly by permeabilising the cells and disrupting vital ion and metabolite homeostasis. This presentation reviews the evidence for this generally accepted mechanism and discusses some recently proposed alternatives. It provides an update on our present knowledge on how the toxin's virulence may be affected by the physical and chemical environment of the target cells, i.e., pH, ionic strength and divalent cations, as well as gut juice and proteolytic enzymes. Our recent results and those from other studies suggest that the rational design of Bt toxins in terms of improved efficiency and delaying of resistance may benefit from strategies taking into account both the physiological response of target cells and the role of their environment.

SYMPOSIUM, Virus Division, Monday 14:00 - 16:00

I: Symposium in honor of Bob Granados.
Insect cells and baculoviruses: 'Pas de Deux'

Symposium. Monday, 14:05. (7)

Developments and significance in insect cell culture

Dwight E. Lynn

INSell Consulting, 247 Lynch Rd, Newcastle, ME 04553

Next year marks a century of use of animal cell culture as a technology. The most significant breakthrough with insect cell culture is the 1962 report by T.D.C. Grace of established cell lines from the emperor gum moth. In the ensuing four and a half decades, nearly 600 cell lines have been described from over 125 insect species, covering most orders and a large number of economically important species. A major emphasis for the development of cell lines has been their use in studies with pathogens and every major insect pathogen group has been studied in cell cultures. Arguably, the most notable use has been with insect viruses, especially the nucleopolyhedroviruses where cell cultures have been instrumental in understanding their biology. While the early investigations focused on the potential use of these viruses as biocontrol agents, techniques developed in the early 1980's resulted in the development of the baculovirus expression vector, an important tool in medicine (pharmacology) and molecular biology (proteomics). In this presentation, I will cover the history of this technology, address unfulfilled objectives and discuss future concerns.

Symposium. Monday, 14:30. (8)

The Peritrophic membrane and the role of enhancins

Ping Wang

Department of Entomology, Cornell University, NYSAES, Geneva, NY 14456

The insect midgut is lined by an invertebrate unique chitin-protein structure, the peritrophic membrane (also known as peritrophic matrix, PM). The physiological importance of PMs has been long recognized and its proposed functions include compartmentalizing the midgut lumen, assisting digestion and protecting the midgut epithelium from physical and chemical damages and microbial infections. The discovery of baculovirus enhancins greatly facilitated the research on the PM structure and formation, its physiological functions and the roles in insect-pathogen interactions. Studies of enhancins have significantly advanced the understanding of the critical function of PMs in protecting insects from microbial infections and demonstrated the potential to target the PM as a novel strategy for insect pest control. With the advances in understanding the biochemistry, structure and function of the PM, the importance of PMs in insects has been increasingly recognized and the potential of targeting the PM for insect control has been explored.

Symposium. Monday, 14:55. (9)

Viral entry in insect cell systems

Gary W. Blissard

Boyce Thompson Institute at Cornell University, Ithaca, NY 14853

In nature, baculovirus infections typically begin in midgut cells and progress into other tissues by production of budded virions (BV). BV subsequently infect many other secondary tissues and this results in an immense amplification of the virus in the animal. Insect cell culture systems have been used extensively to simulate the infection of the secondary tissues where the virus is amplified. Our studies have focused on the major envelope proteins of the BV and their functions in virus interactions with insect cells. I will provide a brief overview of the expression, structure, and functional roles of the GP64 and F envelope proteins. In addition I will present recent data identifying GP64 functional domains that are involved in virus interactions with cellular receptors, membrane fusion, and virus budding from infected cells.

Symposium. Monday, 15:20. (10)

Contributions to virology by Robert R. Granados: reflections bya colleague and friend

Brian A. Federici

Department of Entomology and Interdepartmental Graduate Program in Cell, Molecular and Developmental Biology

University of California, Riverside

Riverside, California 92521

Robert R. Granados, like many outstanding virologists, has made numerous contributions to virology and invertebrate pathology. He initially pioneered studies of plant viruses vectored by insects, namely wound tumor virus and corn stunt virus, showing that these viruses replicated in hemocytes of their homopteran insect vectors, amplifying the virus load that could be transmitted to plants. During this phase of his career, he also took an interest in mycoplasma transmitted by homoptherous insects, showing that these vectors suffered fitness costs when infected by these prokaryotes. Most of his subsequent studies focused on insect viruses and the development of cell lines to study replication and assembly of these viruses, correlating events seen in vitro with those that occur in vivo. Most of us know Bob's research on baculoviruses because it is his most recent. However, soon after leaving his research on plant viruses behind, Bob made important contributions to the virology of cytoplasmic polyhedrosis viruses (CPVs) and entomopoxviruses (EPVs). He showed that CPVs could be grown in insect cell cultures, and later showed the same for certain EPVs. These contributions were important because the infection cycles could be synchronized, and thus the various critical stages of virion assembly identified. While little known today, Bob wrote an excellent review article on entomopoxviruses decades ago showing that mammalian and insect poxviruses shared many physical and biochemical properties, the implication being that they arose from a common ancestral poxvirus. His structural analysis of various insect poxvirus virions carried the implication that mammalian poxviruses may have originated from insect poxviruses, as the virions of dipteran poxviruses most closely resembled those of vac-

cinia and smallpox. Bob's most recent work on nuclear polyhedrosis viruses, granulosis virsues, and insect cell culture will be reviewed by other speakers in this symposium. But here I will note that Bob also carried out pioneering studies on the pathway by which lepidopteran NPVs infect larvae through the midgut epithelium, showing that the budded form of the virion produced in midgut epithelial cells was capable of traversing the basal lamina of this tissue to gain access via the hemolymph to hemocytes and other tissues. While initially controversial, this research and other fundamental contributions to virology by Robert R. Granados that will be highlighted have stood the test of time.

SYMPOSIUM, Bacteria Division, Monday 14:00 - 16:00
Mode Of Action Of Bacillus Thuringensis Cry Toxins
International Forum on Entomopathogenic Nematodes and Symbiotic Bacteria (IFENSB) Session I: Symbiosis

Symposium. Monday, 14:00. (11)

Molecular aspects of Xenorhabdus nematophila-Steinernema carpocapsae association

Heidi Goodrich-Blair
Department of Bacteriology
University of Wisconsin-Madison

Xenorhabdus nematophila is a gamma-proteobacterium mutualistically associated with Steinernema carpocapsae nematodes. The infective juvenile (IJ) stage of the nematode carries X. nematophila into insect hosts, which are killed to obtain nutrients for reproduction. One or a few X. nematophila cells initiate colonization of the developing IJ then proliferate to fully populate this host niche. Colonization assays using X. nematophila metabolic mutants has revealed the presence of some amino acids in the colonization site, but insufficient levels of methionine and threonine to support bacterial growth. We have identified genes necessary for X. nematophila to initiate colonization of the nematode IJ including three, nilA, nilB, and nilC, that encode a ~10-kDa protein of unknown function, a □eta-barrel outer membrane protein, and an outer membrane lipoprotein. Membrane localization suggests that NilA, NilB and NilC function to link an aspect of the external environment to the inner cell. nilA, nilB and nilC are chromosomally linked and coordinately regulated: nilA, B, and C are repressed by the synergistic activity of Lrp and a small, helix-turn-helix containing protein designated NilR, and are positively regulated by the two-component regulator CpxR. We are continuing to investigate the structure, function, and regulation of these colonization factors.

Symposium. Monday, 14:20. (12)

The ins and outs of Photorhabdus luminescens transmission by Heterorhabditis bacteriophora.

Todd Ciche1, Kwi-suk Kim 1, Bettina Kaufman-Daszczuk 1 Ken C. Q. Nguyen 2 and David H. Hall2 .....
1 Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI, USA; 2 The Center for C. elegans Anatomy, Albert Einstein College of Medicine, Bronx, NY, USA

Transmission of the enteric bacterium, Photorhabdus luminescens, by the entomopathogenic nematode, H. bacteriophora, is essential for both partners to infect and reproduce inside insect hosts. We first determined that IJs are formed predominately, if not exclusively, inside the maternal body cavity by endotokia matricida. Therefore, symbiont transmission might be initiated in maternal nematodes. Colonization of maternal nematodes was observed by using pulse-chase type experiments involving the transient exposure of worms to fluorescently labeled symbionts. Shortly after IJ regurgitation of intestinal symbionts, the nematodes become colonized by symbiont cells adherent to the posterior maternal intestine. These cells grow on the maternal intestine before invading the maternal rectal gland cells. The intracellular symbionts divide inside membrane bound vacuoles before being liberated to the pre-IJs developing inside the maternal body cavity. The symbionts then adhere to the pre-IJ pharyngeal-intestinal valve cells (PIVCs), invade the PIVCs, before exiting and colonizing the IJ intestinal lumen. A large scale genetic screen was employed to identify symbiont genes required for transmission. 28 transmission mutants containing disruptions in genes predicted to encode, surface organelles, regulators, metabolic genes and proteins of unknown function were identified. Thus, symbiont transmission is an elaborative and infectious process closely tied to nematode development.

Symposium. Monday, 14:40. (13)

The biosynthesis of stilbene in Photorhabdus luminescens

Joyce, S. A.1, A. O. Brachmann2, G. Schwär2, Lango, L.1, Glazer, I.1, Bode, H. B.2 and David J. Clarke1,3
1Department of Biology and Biochemistry, University of Bath, Bath BA2 7AY, U.K.
2Department of Pharmaceutical Chemistry, Saarbrucken Universuty, GERMANY
3Department of Microbiology, University College Cork, Ireland

Photorhabdus produce 3,5-dihydroxy-4-isopropylstilbene (ST), a small multipotent molecule that inhibits phenoloxidase activity in insect hemolymph and has antimicrobial activity. The genome sequence of Photorhabdus luminescens TT01 contains > 20 genetic loci predicted to be involved in the production of small bioactive molecules although a locus involved in ST production was not obvious. ST is a stilbene, a family of bioactive molecules that are ubiquitous in plants but rarely found in bacteria. The first step in ST production in Photorhabdus requires StlA, a phenylalanine ammonium-lyase (PAL) that catalyses the non-oxidative deamination of phenylalanine to produce cinnamic acid. PAL activity is also required for the first step in stilbene production in plants. In this work we have used both random and targeted mutagenesis to identify genes that are involved in the production of ST from cinnamic acid. These studies have identified additional genetic loci that are required for ST production and reveal a significant interface between amino acid metabolism, fatty acid metabolism and ST biosynthesis. The biochemistry of stilbene production in plants is well characterized and our data show that the biochemical pathway leading to ST production in Photorhabdus is largely unique suggesting independent origins for stilbene production in plants and bacteria.

Symposium. Monday, 15:00. (14)

Flexible gene pool in genomes of the entomopathogenic bacteria, Photorhabdus and Xenorhabdus.

Gaudriault, S.1, Lanois, A.1, Pages, S.1, Roche, D.2, Tamendjari, S.2, Medigue, C.2, Givaudan, A.1
1 : UMR "EMIP" 1133 (INRA-UMII), Université Montpellier II, 34095 MONTPELLIER CEDEX 05, FRANCE
2 : Génoscope/CNRS-UMR 8030, Atelier de Génomique Comparative, 2, rue Gaston Crémieux, 91057 Evry Cedex

Analysis of bacterial genomes structure allows identification of interclonal flexible gene pool that is potentially involved in host or environment adaptation. We search to characterize the interclonal flexible gene pool of Photorhabdus and Xenorhabdus, Gram-negative bacteria, that are in symbiotic associations with entomopathogenic nematodes and pathogenic for insect larvae.
A Photorhabdus DNA microarray was used to compare different species in the Photorhabdus genus. This approach led to the identification of effective interclonal flexible gene pool of Photorhabdus genus, which matches with typical genomic islands and Enterobacteriaceae variable regions. The particular case of the lsr region potentially in the specificity of nematode interaction was detailed.

More recently, sequencing of another Photorhabdus genome (http://www.sanger.ac.uk/Projects/P_asymbiotica/) and two Xenorhabdus genomes (http://www.xenorhabdus.org/) allowed comparative genomics. Characterization of atypical regions and genomic islands common to the two genera or specific to each genus is currently undertaken.

Our lab is also interested in intraclonal genomic plasticity. Indeed, one of the intriguing properties of Photorhabdus and Xenorhabdus genera is the apparition of variants. We studied genomic variation among a set of variants and showed that genomic reduction and amplification occur in Photorhabdus variants. Interestingly, the "intraclonal" flexible gene pool partially matches with the interclonal flexible gene pool.

Symposium. Monday, 15:20. (15)

EPN Phylogeny and Evolution

Byron Adams

Microbiology & Molecular Biology Dept., and Evolutionary Ecology Laboratories, Brigham Young University, Provo UT 84602

Body of Abstract: Entomopathogenic nematodes (EPNs) of the Steinernematidae and Heterorhabditidae are not monophyletic, but likely began to independently explore biotic relationships with arthropods and Gram-negative enteric bacteria (Enterobacteriaceae) by the mid-Paleozoic (approximately 350 million years ago). Their origins were probably not synchronous, and the ages of their respective lineages appear to be significantly different. This could explain, in part, the differences in species richness between the two genera. However, if EPNs in general have been coevolving with their insect hosts for 350 million years, why don't we see numbers of entomopathogenic nematodes equal to the species richness of their insect hosts? In the context of current phylogenetic hypotheses for EPN species, I argue that mechanisms of nematode-bacterium symbiosis, host specificity and virulence exert greater influence on the origin of species in these nematodes than other factors.

CONTRIBUTED PAPERS, Monday 14:00 - 16:00

FUNGI 1

Contributed Paper. Monday, 14:00. (16)

Effect of esterase over-expression on the virulence of Beauveria bassiana infecting the coffee berry borer

Carmenza E. Góngora B1 . Liliana M. Cano M2. María A. Ortega P3 . Lady C. Rosero4

Alvaro L. Gaitan B5

1,2,3,4 Department of Entomology. 5 Department of Phitopathology.

CENICAFE- FNC. National Centre of Coffee Research. PlanAlto. Km 4 Via Antigua Manizales. Chinchina. Caldas. Colombia.Co-financed by the Colombian Agriculture Ministry

To determine the effect of the over-expression of an esterase on the virulence of the entomopathogen Beauveria bassiana infecting the coffee berry borer (CBB) Hypothenemus hampei (Ferrari), protoplasts from the high virulence strain Bb9205 and the low virulence strain Bb9024 were transformed by PEG. The vector pBarGpe1-ste1 was used for the transformations, containing the bar selection gene for resistance to the herbicide glufosinate-ammonium, and the esterase gene ste1 isolated from Metharhizium anisopliae, under the control of the gpdA constitutive promoter. For PCR-positive transformants the copy-number of steI was evaluated using qRT-PCR resulting in three copies for Bb9205-ste1 and multiple insertions for the Bb9024-ste1 transformants. The qualitative esterolytic activity was tested using tween 80 medium and indicated that Bb9205 is a low esterase producer, while no esterases could be detected in Bb9024. The transformants strains showed an increase of esterase activity with respect to the non-transformants controls. Transformed strains from Bb9024 showed a 50% of virulence increase against CBB when compared to the non-transformed controls; however no change in virulence was observed for Bb9205-ste1. These results suggest that esterase production can be a limiting factor in a low-virulence background, such as the one in Bb9024, but that the over-expression of this gene does not influence the virulence in strains that already exhibit this enzyme activity.

Contributed Paper. Monday, 14:15. (17)

Interactions of two natural enemies of Tetranychus evansi, the fungal pathogen Neozygites floridana (Zygomycetes: Entomophthorales) and the predatory mite, Phytoseiulus longipes (Acari: Phytoseiidae)

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Tetranychus evansi is an exotic pest of Solanaceous crops in Africa discovered in Zimbabwe in 1979. Two natural enemies, the predatory mite Phytoseiulus longipes and the fungal pathogen Neozygites floridana are important causes of mortality in T. evansi populations in Brazil. The first part of this study assessed the effects of N. floridana on predation and oviposition of P. longipes fed on N. floridana infected T. evansi and T. urticae. No N. floridana hyphal bodies were found in P. longipes after this feeding, demonstrating that N. floridana is not pathogenic to P. longipes and does not affect its oviposition. The second part of the study investigated the time spent on searching for and consuming of eggs on leaf discs with and without N. floridana capilliconidia. Both the searching and the feeding time on the first egg were similar on leaf discs with and without capilliconidia. When P. longipes was offered the choice of feeding on eggs on leaf disc with or without capilliconidia, the numbers of eggs consumed were not different. The only N. floridana effect observed on P. longipes was reduced egg predation. In addition, increased time spent grooming on leaf discs with capilliconidia was observed. P. longipes was efficient in removing most capilliconidia attached to the body through self-grooming behavior. This suggests that although the predator did not avoid areas with capilliconidia, it detected and removed most capilliconidia attached to the body. Increased grooming may account for the lower egg predation rates.

Key words. Tetranychus evansi, Phytoseiulus longipes, Neozygites floridana, Capilliconidia, Grooming, Hyphal bodies, Classical biological control.

Contributed Paper. Monday, 14:30. (18)

Detection and avoidance of Beauveria bassiana by seven spot ladybirds, Coccinella septempunctata

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Adult seven spot-ladybirds (Coccinella septempunctata) spend the winter months in a dormant state, overwintering in the leaf litter. The insect-pathogenic fungus Beauveria bassiana is a significant winter mortality agent of C. septempuncata . Most ladybirds form aggregations during the winter months however some individuals spend the winter alone. Pathogen theory indicates that transmission and hence mortality will increase as host density increases, for example in aggregations. However, contrary to these theoretical predictions our studies demonstrated that individuals overwintering alone were more likely to succumb to B. bassiana infection than those in aggregations. Through a series of laboratory bioassays we assessed the ability of C. septempunctata to detect the presence of

B. bassiana on soil, leaves and conspecifics. Here we discuss the results and hypothesise that ladybirds can assess and react to environmental cues that relate to mortality risks such as high pathogen density.

Contributed Paper. Monday, 14:45. (19)

Molecular characterisation of Beauveria bassiana isolates obtained from semi-field arenas of overwintering Coccinella septempunctata

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Isolates of Beauveria bassiana were obtained from Coccinella septempunctata overwintering in semi-field arenas at the Genetics Research Center in Cambridge, UK. Beauveria bassiana was also isolated from the semi-field arena soil by Galleria melonella baiting and serial dilution plating on selective media. DNA was extracted from these isolates using ISSR-PCR, and four ISSR primers were used to characterise and to investigate genetic diversity of the isolates at the molecular level. The ISSR-PCR detected a high level of genetic variation among the isolates. Analyses of this data gave indications of intra-specific groupings correlated with source of origin. The grouping of isolates by source of origin, perhaps suggests the overwintering Coccinella septempunctata populations were infected by a sub-section of the native population with superior entomopathogenic abilities.

Contributed Paper. Monday, 15:00. (20)

Myrmica rubra defense against entomopathogenic fungi

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Invasive populations of the European red ant, Myrmica rubra, have become serious pests in some coastal communities in the northeast U.S. and in eastern Canada. High densities of these stinging ants (averaging 1.4 nests/m2) have become a nuisance to home and business owners and are impacting local ecosystems through the displacement of native ant species and promotion of plant feeding homopterans. M. rubra populations in both their native and introduced range succumb to infections by fungi, including Beauveria bassiana and Metarhizium anisopliae. To assess the feasibility of manipulating these fungi for possible biological suppression of the ants, experiments were conducted to explore the behavioral and chemical mechanisms ants use for defense against these fungi.
Allogrooming and necrophoric behaviors were found to be contributing behavioral defenses. Evidence of chemical defenses was also detected and will be discussed.

Contributed Paper. Monday, 15:15. (21)

The invasive coccinellid Harmonia axyridis as an intra-guild predator of the aphid-specific fungus Pandora neoaphidis

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The ladybird Harmonia axyridis is an invasive alien species in many countries and is predicted to have a negative impact on native biodiversity. Intraguild-predation of Pandora neoaphidis by H. axyridis collected from the UK (an invasive population) and Japan (a native population) relative to that of Coccinella septempunctata and C. septempunctata brucki was assessed. Pandora neoaphidis-infected and uninfected Acyrthosiphon pisum were presented as single or choice prey treatments in Petri dish arenas to adult and larval coccinellids that were either starved or unstarved. Overall, predation of uninfected aphids was greater than infected aphids and, when given a choice, a preference for aphids was shown. However, H. axyridis (UK) consumed a greater quantity of fungal cadavers than C. septempunctata, C. septempunctata brucki and H. axyridis (Japan) and showed little preference for uninfected aphids over infected aphids. Harmonia axyridis (UK) is a stronger intraguild predator of P. neoaphidis cadavers than the other coccinellid species and, therefore, may have an impact on the occurrence and persistence of P. neoaphidis. The differences in intraguild predation by H. axyridis collected in the UK and those from Japan suggest that the coccinellids that invaded the UK could have undergone micro-evolution.

Contributed Paper. Monday, 15:30. (22)

Exposure to Beauveria bassiana reduces the fecundity of Harmonia axyridis

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Harmonia axyridis is a predatory coccinellid, native to central and eastern Asia. It has been available in many countries for use as a biological control agent of pest aphids and scale insects. In many of these countries, including the USA, H. axyridis has established. It is now considered an invasive alien species in many countries for a number of reasons including its impact on functional biodiversity. Beauveria bassiana is known to be a natural mortality agent of overwintering ladybirds and is a potential candidate for the biological control of H. axyridis.
In this paper we compare the susceptibility of three species of ladybird, H. axyridis, Coccinella septempunctata and Adalia bipunctata to Beauveria bassiana infection after exposure at three doses (105, 107, 109 spores per ml). In addition we assess the impact of B. bassiana on the fecundity of these three ladybird species. Harmonia axyridis is extremely resistant to B. bassiana infection but even low doses reduce fecundity dramatically. In comparison C. septempunctata and A. bipunctata are highly susceptible to B. bassiana but low doses do not reduce fecundity. We discuss these results in relation to potential for control of H. axyridis using B. bassiana.

Contributed Paper. Monday, 15:45. (23)

A tale of two Continents: Cordyceps in ants

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The parasitic genus Cordyceps is one of the most well known groups of highly specialised insect pathogens and, because of its medicinal qualities for humans, has received significant cultural attention. It is therefore puzzling that its host-parasite evolutionary ecology is poorly known. Ants hold great promise to definitively study the evolutionary ecology of Cordyceps. Here I report on a major project currently underway in our group which is examining Cordyceps evolutionary ecology in Old (Thailand) and New world ants (Panama/Brazil). I will briefly review why Cordyceps are interest-

ing to medics, evolutionary biologists and social insect researchers. Cordyceps adaptively manipulate the behaviour of ants causing them to abandon their colony, ascend plants and die on the underside of leaves in primary rainforest. We counted every dead ant in 540m2 of the forest floor and have demonstrated that ant 'graveyards' exist as hotspots in the forest where ants die en masse (e.g. 26 dead ants/m2). With GIS mapping we have built up a picture of the factors that predict the location of such graveyards. Our detailed collections and spatial accuracy, coupled with bi-monthly collections allows a good understanding of the relevant spatial and temporal changes in fungal population structure. We also relate fungal phenology, spore output and geographic position to arrive at a precise estimate of infection intensity within these graveyards. They are quite literally killing fields.

A highly novel finding was the presence of specialised parasites of Cordyceps. Behaviourally manipulated, and dying ants, are targeted by highly specialized gall midges (Cecidomyiidae) which eat the growing fungus. These flies oviposit on dying ants. Fly damage may promote subsequent infection by a high diversity of hyperparasitic fungi (also Clavicipitaleans). In our study sites substantial diversity of such hyperaparsites exists. It is evident that Cordyceps creates an ecosystem. I will also report briefly on our on-going work into Cordyceps co-evolutionary history with ants, which is making use of excellent herbaria material stretching back 17 years and comprising over 3,000 ants from 59 species of ants.

In the last section of the talk I will report our recent findings of Cordyceps in Panamanian and Brazilian leaf-cutting ants. Here the fungus acts completely different. We also report how we have successfully achieved artificial host-jumping under laboratory conditions across 5 diverse ant species (including one from Europe).

CONTRIBUTED PAPERS, Monday 16:30 - 18:00, *MICROSPORIDIA*

Contributed Paper. Monday, 16:30. (24)

Life cycle characteristics of microsporidia influence their transmission in a lepidopteran host

Dörte Goertz, Gernot Hoch

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We studied possible horizontal transmission pathways of three microsporidian pathogens of Lymantria dispar that differ in life cycle, virulence and tissue specificity. Endoreticulatus schubergi – a midgut parasite – is characterized by low virulence, in contrast to the fat body parasite Vairimorpha disparis and Nosema lymantriae that causes systemic infections. E. schubergi was readily transmitted via spore-ladens feces; a larva released in total 1.6*108 spores until pupation. For V. disparis, spores released from decomposing cadavers were shown to be of major importance for transmission. When a Vairimorpha-infected larva died approximately two weeks after infection, it contained 4.6*109 spores. N. lymantriae utilized both pathways efficiently; an infected larva released in total 2.7*107 spores with feces within a period of about ten days and its cadaver contained 4.9*109 spores. Spores on silk produced by larvae, contact with infected larvae, or exuviae of infected larvae were of minor importance for horizontal transmission in all studied species. While preliminary results indicate that all three microsporidian species might survive winter conditions to infect the next host generation, only two parasites, N. disparis and E. schubergi, were shown to be transmitted vertically. We will discuss all possible transmission pathways of the three microsporidia in the context of their life cycles.

Contributed Paper. Monday, 16:45. (25)

Multiple microsporidia species detection in Agrilus anxius, a congener to emerald ash borer, Agrilus planipennis: - possible biological control agent

George Kyei-Poku, Debbie Gauthier, and Kees Van Frankenhuyzen

Natural Resources Canada, Canadian Forestry Service, Great Lakes Forestry Centre, 1219 Queen Street East, Sault Ste. Marie, Ontario, Canada P6A 2E5

We report for the first time occurrence of natural co-infection of two Nosema spp. and a Cystosporogenes sp. in the bronze birch borer, Agrilus anxius Gory, an indigenous congener to the emerald ash borer (EAB), Agrilus planipennis Fairmaire, an introduced exotic species

Populations of A. planipennis and A. anxius collected from various locations in Canada were screened for the presence of microsporidia using PCR-based detection. This survey was part a project to establish an entomopathogen database to identify potential biological control agents for managing A. planipennis infestations in North America.

Sequencing and phylogenetic analysis revealed 2 distinct Nosema species and a Cystosporogenes sp. in A. anxius. On the basis of partial SSU rRNA sequence information, we developed a discriminatory PCR-RFLP test to differentiate between the Nosema spp. and Cystosporogenes sp infecting A. anxius. This screen allows rapid detection and discrimination of microsporidia in natural field populations. The occurrence of two vertically transmitted parasites within a population has implications for our understanding of parasite-host relationships in the field and we discuss factors affecting the dynamics of parasite-parasite competition and coexistence.

Contributed Paper. Monday, 17:00. (27)

Rapid molecular diagnosis of nosema disease in honeybee

Wei-Fone Huang and Chung-Hsiung Wang

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Nosema ceranae has been reported in the Apis mellifera and found in many areas in the world. N. ceranae is similar to N. apis in morphology and easily confused using microscopic examination, the primary diagnostic method. In order to obtain a more accurate diagnosis, a multiplex PCR method was designed. For this method, specific gene fragments were concurrently amplified from N. apis and N. ceranae. The amplicons can be easily detected and distinguished after agarose gel electrophoresis. This molecular diagnosis could be done within few hours and facilitates pathogen identification for large-scale survey of honeybee nosema disease.

Contributed Paper. Monday, 17:15. (28)

In vitro propagation of a microsporidian isolate (Nosema sp.) from yellow butterfly, Eurema blanda

Yi-Chun, Tsai, Chih-Yuan, Wang, Chung-Hsiung, Wang

Department of Entomology, National Taiwan University, Taipei 106, Taiwan

A microsporidium was isolated from the larvae of yellow butterfly (Eurema blanda). This isolate possesses the molecular and morphological characteristics of the genus Nosema. The organization of the rRNA genes is LSUrRNA-ITS-SSUrRNA-IGS-5S. In phylogenic analysis based on the sequences of rRNA genes, this isolate is closely related to N. plutellae, N. spodopterae, and N. bombycis. The in vitro propagation of this isolate in NTU LY, Lymantria xylina, cell line was successfully established. The microsporidum multiply well in the cytoplasm of NTU LY cells but frequently a few microsporidia were found in the nuclei of the infected LY cells. About 14 days after inoculation, some developing stages of the microsporidium were extruded from the infected cells and became suspended in the culture medium. These extruded developing microsporidia

were infective and could invade newly added LY cells. With in vitro propagation, the functional genomic study of this isolate will be undertaken in our ongoing studies.

Contributed Paper. Monday, 17:30. (28,05)

Phylogeny and classification of the phylum Microsporidia

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The present classification of the Microsporidia is based largely on ultrastructural (spore shape, number of coils in the polar filament, nuclear condition etc.) and developmental (spores/sporont, presence or absence of an alternate host etc.) character states. The consistency of these character states in defining taxa has been called into question. Comparative molecular data allows us to obtain many more characters with which to examine evolutionary relationships. I present here a molecular phylogeny for the Microsporidia, discuss the ontogeny of the traditional characters and suggest a plan for developing an acceptable classification scheme for the Microsporidia.

Bacteria Division Workshop, Monday 21:00 - 22:00

So Many Strains, so Few Products! Opportunities and Constraints to Commercial Development of new Bt Products.

Workshop. Monday, 21:00. (28,1)

The Regulatory Challenge

Dr. Brian Belliveau, Health Evaluation Directorate, Pest Management Regulatory Agency, Health Canada, Ottawa, Ontario, CANADA.

Regulatory authorities in Canada, the United States, and the European Union have all recently conducted a re-evaluation of existing subspecies of Bacillus thuringiensis, including (but not limited to) strains of Bt kurstaki, israelensis and tenebrionis to determine their continued acceptability as microbial insecticides. Even though Bt is one of the oldest, and arguably most widely applied, microbial pest control agents used today in agriculture and forestry, regulators are legislatively required to periodically re-examine the safety of pesticides to ensure that the supporting test data and risk assessments are consistent with current standards. This re-evaluation exercise is also an opportunity for regulators to consider and evaluate new research that may call into question the human health or environmental safety of a pesticide product. Regulatory authorities have depended largely on test data generated by registrants to assess the pathogenicity and toxicity potential of Bt in vivo, as well as the extensive body of peer-reviewed scientific literature to assess the overall impact of Bt on human health and the environment. The outcome of these re-evaluation exercises has identified specific issues that have presented challenges to the regulatory community. These unique challenges will be discussed in a global regulatory context, the focus of which concerns the significance, and implication, of certain B. cereus-type enterotoxins in existing registered strains as well as new, uncommercialized strains of Bt.

Workshop. Monday, 21:15. (28,2)

Why companies don't pick up new strains

Workshop. Monday, 21:30. (28,3)

Natural variation in Bt Cry toxins

Neil Crickmore

School of Life Sciences, University of Sussex, UK

Since the first Cry toxins were cloned in the 1980s almost 200 new insecticidal proteins have been characterised from strains of Bacillus thuringiensis. As well as the Cry toxins we now have Cyts, Vips, Sips and parasporins as well as some which as yet don't fall into any convenient category. No longer do the Cry toxins represent a class of proteins unique to this species of bacterium as proteins are found within the Bt crystal that look and to some extent behave like toxins from mammalian pathogens. This presentation will consider the implications of the discovery of all these toxins for the development of new commercial Bt products.

Workshop. Monday, 21:45. (28,4)

Criteria for selection and use of native Bt strains

Rose Monnerat - EMBRAPA Recursos Genéticos e Biotecnologia, SAIN-Parque Rural, Caixa Postal: 02372, 70770-900-Brasilia-DF, Brasil, rose@cenargen.embrapa.br

The pests control is a fundamental aspect on the agricultural production and in the reduction of diseases transmited by vectors. Only a small proportion of the agrochemical market (2%) is based on the using of biological insecticides, where the Bacillus thuringiensis represents 90-95%. It is estimated that this utilization will increase as long as the more rigorous legislations of environmental protection are accepted and the more effective and cheaper products are launched. There is a great quantity of producers involved in the production and formulation of B. thuringiensis in many countries such as the United States, Belgium, Switzerland and France. A survey recently accomplished pointed that there are about 60 products made from this bacterium, being still in development new formulations for special uses, such as environments with great incidence of solar radiation, irrigated fields and aquatic environments. A very important aspect in the development of new bio-insecticides is the discovery of strains with higher activity or more adapted to the environmental conditions where these products will be used. Some countries such as Brazil have been using these new strains as a base of biological products against mosquitoes and caterpillars. The criteria for the selection of Bt strains should be based on their toxicity, ability of growing in very cheap medias and the power to kill insects.

Virus Division Business Meeting and Workshop. Monday, 20:00

Polydnavirus Phylogeny and Taxonomy

Workshop. Monday, 20:00 (28,5)

Polydnavirus taxonomy and the ICTV

Peter Krell

Department of Molecular and Cellular Biology, University of Guelph, Guelph, ON Canada

The nuclear secretory particles first described in 1975 and now known as polydnaviruses, were first "officially" classified and named as members of a proposed Subgroup D of the Family Baculoviridae, Genus Baculovirus. in 1982 in the Classification and Nomenclature of Viruses in the Fourth Report of the International Committee on Taxonomy of Viruses (REF Matthews Editor). Subgroup D consisted of type species D1 Hyposoter exiguae virus and type species D2 Apantales melanoscelus virus. Their placement in the Baculoviridae was based largely on their morphological similarity to baculoviruses. Following the pioneering work of researchers at Canada's Dalhousie University on their structural, molecular and replication characteristics, it was clear that they needed their own family in part because of their highly unusual "polydisperse" dsDNA genome. By the Fifth Report of the ICTV in 1985, they became of age, were recognized as polydnaviruses and were allotted their own Family, the Polydnaviridae consisting of two genera, Ichnovirus and Bracovirus along with two new type species. The proponents had to convince the International Committee on Taxonomy of Viruses (ICTV), a division of the International Union of Microbiological Societies (IUMS) that they merited such recognition. However, even having them officially recognized in this manner was no mean feat. Prior to that the proposal was formulated for wider distribution and comment (Intervirology 21:1-4). It helped that at the time Don Stoltz was the first Chair of the Polydnavirus study Group and Max Summers, as Chair of the Invertebrate Virus Subcommittee, presented the Polyd-

naviridae proposal to the ICTV. Since then the family and its members have undergone reiterations up to the 2005 VIIIth ICTV report and during the interim more species have been added. Although less authoritarian than its predecessor, the current ICTV is still fairly demanding in approving addition of new species and generating or changing virus taxa. The ICTV does not take taxonomic changes lightly and needs to be persuaded that changes are warranted. It is in this context that any new proposals for changes to the taxonomy of Polydnaviridae must be clearly presented and justified and shown to be supported by the wider polydnavirus community.

Monday ***16:30 - 18:30***

POSTERS - I

BACTERIA

Poster / Bacteria. B-01

Three Bt-resistant populations of Plutella xylostella with diverse phenotypes share a common resistance locus.

Ali H. Sayyed and Neil Crickmore
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Crop plants engineered to produce insecticidal toxins derived from the bacterium Bacillus thuringiensis (Bt) are being grown on millions of hectares. The high selection pressure exerted by Bt cultivars could lead to the development of resistance in the field. In this study we tested the hypothesis that separate populations arrive at the same genetic solution when faced with similar selections by comparing resistant strains of diamondback moth isolated from the Serdang region (SERD4), the Karak region (Karak) and the Keluang region (Keluang) of Malaysia. The SERD4 population is multigenic with an incompletely dominant mode of inheritance of resistance. Keluang is also autosomal and incompletely dominant whilst Karak presents a single, recessive, autosomal resistance gene. The Karak population has a fitness cost associated with the resistance phenoype whereas both SERD4 and Kaluang have a fitness advantage. Despite dissimilarities between populations, complementation studies revealed that a major resistance locus is shared between SERD4, Karak and Keluang populations. Results of biochemical studies showed that the loss of binding affinity of the midgut to Cry1Ac/Cry1Ab is a major mechanism of resistance in parents and F1 crosses (Karak x SERD4, Karak x Keluang and SERD4 x Keluang).

Poster / Bacteria. B-02

Study of the mechanism of resistance to Bacillus thuringiensis Cry3A toxin in a natural population of the leaf beetle, Chrysomela tremulae (Coleoptera: Chrysomelidae).

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3 Institut National de la Recherche Agronomique, UMR CBGP, Campus International de Baillarguet, 34988 Montferrier-sur-Lez.

Cry toxins of Bacillus thuringiensis (Bt) represent a class of bioinsecticides that are attractive alternatives to broad-spectrum chemicals. The high specificity, potency, and environmental safety of Cry toxins have led to their wide use in sprayable Bt formulations or transgenic crops. However, evolution of resistance is the main threat to the widespread commercial use of Bt toxins. In a field population of the leaf beetle Chrysomela tremulae, highly resistant individuals able to survive on transgenic Cry3A-Bt poplar were identified. Genetic analyses showed that resistance to Cry3A toxin was almost completely recessive and conferred by a single autosomal gene. To get more insight in the identification of the resistance mechanism, in vitro binding experiments using a biotinylated derivative of Cry3A on membranes prepared from midguts (BBMV) of susceptible and resistant L3 larvae C. tremulae were done. Results show that Cry3A binds specifically on BBMV isolated from susceptible larvae while almost no binding can be detected in the case of BBMV from resistant ones suggesting that an alteration in the binding site is responsible for such resistance. Following work will focus on the isolation and characterization of the receptor using molecular and biochemical techniques, thus allowing the first characterization of a Cry toxin receptor in coleopteran insects.

Poster / Bacteria. B-03

Characterization of intracellular signaling in mosquitoes in response to Bacillus thuringiensis subspecies israelensis toxins

Angeles Cancino-Rodezno 1, Roberto Villaseñor 2, Mario Soberón, Alejandra Bravo
1.Instituto de Biotecnologia UNAM, 2.bachelor student

Dengue and malaria are human diseases produced by arbovirus and protozoans from the genus Plasmodium, respectively. Both pathogens require mosquito vectors in order to access their hosts. Ever since these mosquitoes were identified as vectors the control measures against these diseases have focused on mosquitoes.

The use of bioinsecticides that are specifically directed against mosquitoes and have no collateral effects on other organisms represents a promising alternative. Gram-positive bacterium Bacillus thuringiensis (Bt) subspecies israelensis (Bti) produces protein crystals that are highly toxical for the larvae of the mosquitoes belonging to the genuses Aedes and Anopheles. Therefore, Bt toxins are considered to be potential agents for the control of vector insects involved in human diseases.

Bt distinguishes itself from other bacilli by its ability to synthesize proteins Cry and Cyt, which are pore-forming toxins (PFTs) with entomopathogenic properties whose general mechanism is as following: Cry proteins show a sequential binding mechanism to specific receptors. One of these receptors is anchored by a glycosyl phosphatidyl inositol (GPI) bridge into lipid rafts. It has been proposed that a high concentration of the toxin in the lipid rafts has a dual effect: it induces both the formation of pores and the activation of intracellular signaling pathways. Finally, as a consequence of the flow of water and ions through the pore that has formed in the membrane, the cells are lysed and the insect dies. However, it has been recently suggested that invertebrates are able to acquire tolerance and/or resistance against Bt through defensive responses initiated downstream of the initial binding of the Cry toxins. Using functional proteomics, it is possible to analyze - both globally and differentially - the expression of proteins in two separate samples. The information thus obtained enables the identification of the proteins involved in a particular condition (for example, intoxication with Cry). Since the larvae of A aegypti and A. gambiae are susceptible to Bti toxins, studies on the response to these proteins in mosquitoes shall contribute information for the control of these dipterans.

Poster / Bacteria. B-04

Involvement of a Colorado potato beetle membrane associated metalloprotease on Cry3Aa Bacillus thuringiensis mode of action

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Insect proteases are implicated in Bacillus thuringiensis insecticidal proteins mode of action determining toxin specificity and sensitivity.

In this study, a Colorado potato beetle (CPB) ADAM metalloprotease has been involved in the recognition and cleavage of Cry3Aa. We have found that CPB BBMV specifically catalyzed Cry3Aa cleavage in accessible regions of domain III. This cleavage resulted in potentially active toxin degradation, since proteolysis inhibition by a peptide representing one segment of proteolysis in domain III correlated with enhanced pore formation. Cry3Aa proteolysis was specifically inhibited by the metalloprotease inhibitors acetohydroxamic acid and 1,10-phenantroline implicating CPB membrane associated metalloproteases in Cry3Aa cleavage. By ligand blot analysis, we identified a Cry3Aa toxin binding molecule that on the basis of sequence homology by N-terminal analysis, cleavage substrate and recognition site specificity is an ADAM 10 metalloprotease homolog that could be responsible of Cry3Aa proteolysis, because cleavage of an ADAM fluorogenic substrate by CPB BBMV was competed by Cry3Aa. A recognition motif, also shared by other ADAM 10 substrates, was identified in the stretch from amino acid 342-349 within loop I of Cry3Aa domain II. A peptide (pep-rec) representative of this loop effectively prevented Cry3Aa interaction with the CPB BBMV membrane and nearly abolished pore formation, evidencing the functional significance of Cry3Aa-ADAM interaction in relation to this toxin mode of action.

Poster / Bacteria. B-05

Ultrastructure of Culex quinquefasciatus midgut cells from susceptible and Bacillus sphaericus-resistant larvae: morphology and cytopathological effects

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The activity of Bacillus sphaericus (Bsp) towards Culex quinquefasciatus relies on the binding of the binary toxin to the α-glucosidase Cqm1, a midgut membrane-bound receptor. After binding the toxin provokes cytophatological effects and larval death. This work aimed to describe the effects of the Bin toxin and compare the cell morphology using a susceptible and a Bsp-resistant colony, lacking the receptor Cqm1. To evaluate the effects of Bin toxin, larvae were treated and midguts were processed for transmission electron microscopy. The major effects in cells from susceptible larvae were microvilli disruption and mitochondrial distention, while resistant larvae showed only minor alterations. The cell morphology of aging larvae was compared in samples fixed at 6 and 48h after the 4th instar moult. Some electron lucent inclusions were detected in cells of susceptible larvae fixed at 48h. Resistant larvae fixed at 6h already showed a high number of those inclusions and at 48h they were even more abundant. The inclusions had a lipid nature and their amount in resistant larvae could be related with the absence of the receptor Cqm1, since this molecule is a maltase that plays an important role in the metabolic pathway.

Poster / Bacteria. B-06

Immunolocalization of the gypsy moth Bacillus thuringiensis toxin receptor by electron microscopy

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Bacillus thuringiensis (Bt) produces insecticidal crystal (Cry) proteins, which interact with receptors on the midgut epithelial cells of susceptible insects. Immunolocalization of Bt toxin binding sites in lepidopteran insects has been previously reported: the Bt toxins accumulate at the apical microvilli. In this study, we used an immunogold electron microscopic procedure to study the distribution of BTR-270 in the gut tissue of 3rd and 5th instar gypsy moth larvae. BTR-270 is a high molecular weight glyconjugate, which interacts with Cry1A and Cry2A toxins. The affinities of the toxins to BTR-270 correlate with their respective toxicities. The distribution of the immunogold label for BTR-270 was essentially similar to that reported for the localization of Bt toxin binding sites in lepidopteran larvae midgut tissue. The label was concentrated at the microvilli on the brush border membrane of midgut epithelial cells. Little or no labeling was observed in goblet cells or in the cytoplasm, confirming that BTR-270 is an intrinsic and specific component of gypsy moth brush border membrane microvilli.

Poster / Bacteria. B-07

Overcoming microbial insecticide resistance in blackfly larvae by combinations of them.

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In order to overcome resistance to microbial insecticides in blackfly larvae as it occurs with Bacillus sphaericus in mosquitoes (Mulla et al., 2003), we tested combinations of commercial formulations of Bacillus thuringiensis serovar. Israelensis (Vectobac-AS) (Bti), Bacillus sphaericus (Vectolex WDG) (Bsph), and a home-made preparation of Clostridium bifermentans serovar. Malaysia (strain CH18 from Institut Pasteur) (Cbm). We first determined the lethal doses of each microbial insecticide separately on summer and winter blackfly larvae. For combinations, we chose a concentration corresponding to little less than LD10%. The LD10% were respectively 3, 3000, and 165 mg/L for Bti, Bsph, and Cbm on winter larvae, and 1, 3000, and 16.5 mg/L on summer larvae. The combinations of Bti and Bsph always gave antagonistic effects on winter or summer larvae. Only a mixture of Clostridium bifermentans serovar. Malaysia with one of the two others produced for some combinations additive or synergistic effects: Bti 1 mg/L or Bsph 1000 mg/L + Cbm 8.25 mg/L on winter larvae, and Bti 2 mg/L or Bsph 2000 mg/L + Cbm 8.25 or 16.25 mg/L on summer larvae. We also tried combinations of the three microbial insecticides and obtained antagonistic effects.

Poster / Bacteria. B-08

Agricultural BioTech Regulatory Network

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The use of genetic engineering to improve agricultural production and food security recently marked its 10th anniversary by surpassing the one-billionth acre planted in 21 countries. Over the past decade, farmers have increased their plantings of biotech crops by double-digit growth rates every year making modern biotechnology one of the most rapidly adopted in the history of agriculture.

At the same time, over 130 countries have adopted an international treaty governing the movement of products of modern biotechnology and are in some phase of establishing national requirements for use and import of biotech crops. This means technology developers and product marketers face a formidable array of product registration requirements to enable commercialization. Not recognizing these requirements in advance has inevitably led to delays and multiplication of costly studies.

To address a growing demand, we established a global network of experienced regulatory professionals to guide technology developers to meet the challenges of pre-market approvals for genetically engineered plants and plant products. Working cooperatively, the ABTR Network (http://www.abtrnetwork.com) serves the agricultural biotechnology industry from product conception through commercialization. It is a unique network offering academic groups, development countries and start-up business the same quality and

diversity of regulatory support that today only large multi-national corporations have in place.

Poster / Bacteria. B-09

Why are Bacillus thuringiensis formulations ineffective against Spodoptera litura feeding on strawberry leaves?

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Formulations of the entomopathogenic bacterium Bacillus thuringiensis (Bt) are among the most widely used biopesticides in the world. The majority of Bt products are based on two lepidopteran-active strains, B. thuringiensis var aizawai and B. t. var kurstaki. Previous reports have shown that Bt products have low efficacy against pests in strawberry fields, although the mechanism underlying this phenomenon are unclear. In the present investigation, we found that Spodoptera litura larvae fed on strawberry leaves were significantly more resistant to Bt products than were larvae fed on cabbage leaves. Crystal toxins of B. thuringiensis were incubated in vitro with digestive juice of S. litura and homogenates of strawberry or cabbage leaves, and then subjected to SDS-PAGE. These experiments showed that strawberry leaves inhibit solubilization and activation of the crystal toxins. Addition of strawberry leaf homogenate decreased the pH of S. litura digestive juice, perhaps because the polyphenol content of strawberry leaves is significantly higher than that of cabbage leaves. Our results suggest that when larvae ingest crystal toxins together with strawberry leaves, the reduction of digestive juice pH inhibits solubilization-dependent activation of crystal toxins, thereby accounting for the low efficacy of Bt products in strawberry fields.

Poster / Bacteria. B-10

Entomopathogenic and non pathogenic bacterial antigens affect Malacosoma disstria (Lepidoptera: Lasiocampidae) larval hemocytes in vivo and in vitro

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Malacosoma disstria larvae are a pest of deciduous trees. Little is known on the interaction of bacteria with the immediate hemocytic antimicrobial responses of these insects. Incubating dead Xenorhabdus nematophila and Bacillus subtilis with a mixture of serum-free granular cells and plasmatocytes in vitro revealed differential bacterial-hemocyte adhesion and differential discharge of lysozyme and phenoloxidase but not total protein. Although active phenoloxidase adhered equally to both bacterial species, X. nematophila limited enzyme activation whereas B. subtilis enhanced activation. Serum with active phenoloxidase (as opposed to tropolone-inhibited phenoloxidase) and purified insect lysozyme increased bacterial-hemocyte adhesion of both bacterial species. However, initial binding of apolipophorin-III-like protein to both bacteria increased granular cell levels with adherent bacteria while lowering the plasmatocyte levels with adhering procaryotes. Apolipophorin-III-like protein also increased lysozyme and phenoloxidase activities. Although B. subtilis in vivo elicited a nodulation-based decline in total hemocyte counts and did not affect hemocyte viability, dead X. nematophila elevated hemocyte counts and damaged the hemocytes as lipopolysaccharide levels increased and X. nematophila emerged into the hemolymph. The effects of lipopolysaccharide from X. nematophila, and lipoteichoic acid from Bacillus subtilis on larval M. disstria cellular and humoral immune factors were also determined.

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Poster / Bacteria. B-11

Conjugative relationship among Bacillus thuringiensis and Bacillus cereus strains

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Bacillus thuringiensis and Bacillus cereus display considerable genetic similarity despite their diverse niches. Both species are members of the Bacillus cereus species group. B. thuringiensis subsp. kurstaki KT0 harboring a pHT73-EmR plasmid containing the cry1Ac gene, which codes for an insecticidal toxin, and a resistance marker for erithromycin, was used for an analysis of conjugation preference when the recipient strain was either B. thuringiensis or B. cereus. Experiments took place both in vitro and in vivo in Bombyx mori larvae. The results of the triparental conjugation indicated that there is no preferential pattern in plasmid exchange between B. thuringiensis and B. cereus using strains that were suitable in biparental conjugation. In B. mori larvae, B. cereus recipient strains improved its conjugative performance, being equal to the B. thuringiensis strains. The pHT73-EmR plasmid showed stability after 100 generations in all the exconjugants.

Poster / Bacteria. B-12

Isolation of mosquitocidal and non-mosquitocidal Bacillus sphaericus

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The Gram-positive bacterium, Bacillus sphaericus, is so-named because it produces spherical spores during sporulation. Since the first B. sphaericus strain active against mosquito larvae was reported in 1965, many mosquitocidal strains of B. sphaericus have been isolated worldwide. Mosquitocidal B. sphaericus has a narrower target spectrum than B. thuringiensis subsp. israelensis, being only effective against mosquitoes. Even against these, its activity is poor against several important species, such as Aedes aegypti. Nevertheless, it is slightly more toxic than B. thuringiensis subsp. israelensis to some Culex species, and it has better residual activity in polluted waters. However, unlike B. thuringiensis subsp. israelensis, high mosquitocidal activity of B. sphaericus is primarily due to a single toxin, Bin and, as a consequence, field populations of mosquitoes develop resistance rapidly where it is used intensively. In addition, introduced mosquitocidal genes to enhance toxicity have not been expressed stably in most widely used mosquitocidal strains such as 2362. Therefore, to isolate mosquitocidal B. sphaericus with different toxin component and better efficacy, soil and mud samples from various mosquito habitats in Florida are having been screened. In the present study, isolates showing high and no toxicity against Cx. quinquefasciatus 4th instars were characterized.

Poster / Bacteria. B-13

Isolation and characterization of a novel Bacillus thuringiensis strain expressing a novel crystal protein with cytocidal activity against human cancer cells

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The Bacillus thuringiensis strain M15 was isolated from dead two-spotted spider mites (Tetranychus urticae Koch; Arthropoda: Arachnida: Tetranychidae). It is an autoagglutination-positive strain and is therefore non-serotypeable. A sporulated culture produces a roughly spherical parasporal inclusion body, the crystal, tightly coupled to the spore. Although the crystal appears to be composed of at least two major polypeptides of 86- and 79 kDa as estimated by SDS-polyacrylamide gel electrophoresis, Southern hybridization indicates that the corresponding crystal protein gene is likely present in only one copy. The crystal protein gene was cloned and, based on nucleotide sequence homology with an orthologous cry31Aa1 gene, assigned the name cry31Aa2.

Although initially isolated from spider mites, B. thuringiensis M15 is non-toxic to spider mites and it does not produce the wide spectrum β-exotoxin. Assays on mammalian cells, however, reveal that Cry31Aa2, when cleaved with trypsin, is cytocidal to some human cancer cells, most notably HepG2, Hepatocyte cancer cells, but not to normal human cells. No cytocidal activity was induced after protease treatment of Cry31Aa2 with either chymotrypsin or proteinase K. Trypsin, chymotrypsin and proteinase K cleavage sites were determined.

Poster / Bacteria. B-14

Systemic Use of Bacillus thuringiensis, a New Alternative for the Control of Insects Pest

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The bacterium Bacillus thuringiensis is the mainstay of biological control of insect pests of crop plants, either through direct spraying onto plants or by the incorporation of its toxin genes into transgenic crops for a recent appraisal of this field. It is commonly found as a soil bacterium and has been isolated from the surfaces of plant leaves. Insect mortality results from highly potent and insect specific protein toxins that are produced during sporulation. Despite widespread use over several decades few accounts of endophytic Bt appear in the literature, none report distribution beyond the stem and no resultant biological activity in leaves as a result of endophytic Bt has ever been discovered. In this report, we demonstrate that B. thuringiensis appear to exist as natural endophytes and that Bt applied to the roots are transported through the plant where they can accumulate in leaves at a level sufficient to produce significant insect mortality. Studies for the inoculation and colonization of the B. thuringiensis on the plant may point to a new way of insect control, never tested before with this bacterium, reducing, this way, the use of insecticides and their undesirable consequences.

Poster / Bacteria. B-15

Production and efficacy of an experimental tablet formulation for Aedes aegypti control using a Bacillus thuringiensis var israelensis asporogenic mutant strain

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Aedes aegypti is the main vector of dengue and dengue haemorragic fever in the Americas. Chemical insecticides and Bacillus thuringiensis var israelensis (Bti) based formulations have been used for larval control; however, the use of living spores could be a limitation of the biological products. The development of an asporogenic strain of Bti has been achieved and production of the active ingredient was scaled up from 20 to 200 L. The active ingredient was obtained using tangential flow filtration and spray drier systems. Tablet formulations were developed with Bti and asporogenic Bti strains using inert ingredients employed in the pharmaceutical industry. Field simulated experiments were set with 2 treatments of each formulation in 70 L containers with 50 L of water. Treatments of 3 and 7 tablets per container were set by triplicate with a negative control treatment. Twice a week 30 first instar larvae were added to each container and every day the number of pupae were counted, withdrawn and data was registered as the percentage of pupae reduction. After 14 weeks, the percentage of pupae reduction varied between 36.2 and 99.1. No significant differences have been found between the Bti treatments or between doses used.

Poster / Bacteria. B-16

Characterization of sporulation histidine kinases from Bacillus thuringiensis

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Bacillus thuringiensis (Bt) is a spore- forming bacterial host for a variety of Cry proteins, which are produced during sporulation. The Cry proteins are the most widely used bioinsecticide to manage agricultural pests. The initiation of sporulation in B. subtilis and most likely in aerobic Bacillus is controlled by the phosphorelay signal transduction system. The signals that initiate phosphorelay are percibed by five different histidine kinases (HK) that are codificated independently in the genome. HKs are homodimeric proteins that act as membrane proteins sensing environmental and metabolics signals to trigger entry into sporulation. Bioinformatic analysis have shown that Bt has 13 different HKs, some of which can be involved in the regulation of sporulation. The goal of this research is to identify Bt HKs that have a role in sporulation. The experimental approach involves making null mutants from seven out of thirteen independent HKs identified by sequence analysis on the Bt genome. At the present time null mutants are being constructed. Mutants will be analyzed for spore formation, sporulation time, spore numbers and compared against the wild type strain. Mutant strains with defects in sporulation will be further analyzed for expression of early sporulation genes spoIIA y spoIIE and spoIIG.

Poster / Bacteria. B-17

Functional studies of the Insecticidal Toxin Complexes of Photorhabdus luminescens and Yersinia

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Photorhabdus luminescens is a gram-negative bacteria insect pathogen, which lives in the gut of entomothogenic nematodes from the family Heterohabditidae. Upon invasion of the insect host, the nematode regurgitates the bacteria, which releases a plethora of virulence factors to aid killing and bioconversion of the insect.

One of the dominant secretion factors are the high molecular weight insecticidal toxin complex (Tc's) proteins, which have been shown to be orally and injectably toxic. Originally isolated from strain W14, these complexes are made from 4 loci; tca, tcb, tcc and tcd, the genes within these loci labelled according to their order e.g. tcdA, tcbB, tccC. Significant homology is observed between the loci and previous work has shown that three components are required for full toxicity, the tcdA-like [A], the tcbB-like [B] and tccC-like [C] genes.

Interestingly these Tc's are seen in a variety of gram-negative pathogenic bacteria including Yersinia, suggesting an evolving function or targets for these proteins directed towards insect and/or mammalian hosts.

We are currently using heterologous expression of the individual polypeptides, from both Photorhabdus and Yersinia, in a mammalian cell culture model system in order to ascribe their function. Here we report the emerging mode of action for the Tc's.

Poster / Bacteria. B-18

Genetic variation of Helicoverpa armigera populations around the cotton growing area in southern Spain as revealed by amplified fragment length polymorphism (AFLP).

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Amplified fragment length polymorphisms (AFLPs) were used to determine the population genetic structure and variation of Helicoverpa armigera in the cotton growing region in the south of Spain. Individuals were collected, before and after the cotton harvest in 2003 and 2004, from three separate areas where cotton is planted (Seville), and from strawberry greenhouses (Huelva), outside the cotton growing area. A neighbour-joining phylogram of the 19 populations showed a structure that clearly separated populations by season. Results of population genetic structure revealed high levels of diversity within all populations studied and low levels of genetic diversity among all populations. Furthermore, differences among groups were found significant only when populations were grouped by means of season irrespective their origin. The results obtained in this study in H. armigera populations in the south of Spain indicate that populations are not defined by their origin and individuals from one place are completely replaced season after season by insects from other crops or fields after insecticide treatments. This data provide support for considering these populations away from migration-drift equilibrium and all populations of insects could be considered as a single metapopulation.

Poster / Bacteria. B-19

16S ribosomal RNA based assessment of the taxonomic position of Rickettsiella melolonthae

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We report the determination of a 16S rRNA encoding sequence (/ rrs/ gene) of /Rickettsiella melolonthae/, an intracellularly multiplying bacterial pathogen of the two European cockchafer species, /Melolontha melolontha/ (Linnaeus, 1758) and /M. hippocastani/ (Fabricius, 1801) (Coleoptera: Scarabaeidae). /R. melolonthae/ is currently classified as a pathotype of the nomenclatural type species /Rickettsiella popilliae/. Previous sequencing of a 16S rRNA gene from a different species, /Rickettsiella grylli,/ has motivated the transfer of the genus /Rickettsiella/ from the a-proteobacterial family /Rickettsiaceae/ to the g-proteobacterial family /Coxiellaceae/. Comparisons of the /R. melolonthae rrs/ gene with homologous bacterial sequences by the Maximum Parsimony, Minimum Evolution, Maximum Likelihood, and Neighbor Joining methods support this reassignment of the genus, but substantiate inconsistencies in its internal organization. In particular, the existing delineation of /Rickettsiella /species and the claimed synonymy of /R. melolonthae/ with /R. popilliae/ are not simultaneously consistent with our findings. Our results are suggestive of the classification of /R. grylli/ and /R. melolonthae/ in the same species.

Poster / Bacteria. B-20

Molecular characterization of a novel mosquitocidal crystal protein, Cry50A, from a Japanese isolate of Bacillus thuringiensis serovar sotto strain

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A novel crystal protein gene, cry50A, was cloned from a mosquitocidal strain of Bacillus thuringiensis serovar sotto. The cry50A gene was 2,040 bp in length and encoded a molecular mass of 77,002 Da. The Cry50A possessed five conserved blocks commonly found in the existing Cry proteins. The amino acid sequence of Cry50A showed 70% similarity to those of the N-termini of Cry4 proteins. When expressed in crystal-negative B. thuringiensis strain BFR1, the protein accumulated as inclusions. The purified inclusions exhibited insecticidal activity against second instar larvae of Aedes aegypti.

Poster / Bacteria. B-21

Cloning and expression of the potential receptor of Bacillus sphaericus binary toxin in Anopheles gambiae

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The action of the major insecticidal factor of Bacillus sphaericus, the binary (Bin) toxin, depends on the binding to specific membrane-bound receptors from larvae midgut. The receptor in Culex quinquefasciatus larvae is a 60-kDa α-glucosidase (Cqm1). Analysis on the GenBank revealed that the maltase Agm1 from Anopheles gambiae has high similarity to Cqm1. The goal of this work was to investigate the potential of Agm1 to be the receptor of the Bin toxin. PCR reactions were performed and the gene fragment of 870 base-pair that codes the 45-kDa N-terminal region of Agm1 was obtained. The fragment was cloned in the vector pRSETc, followed by the expression of the recombinant protein (Rec-Agm1) in Escherichia coli. Enzymatic assays showed that Rec-Agm1displayed α-glucosidase activity and in vitro affinity assays showed that this protein had the ability to specifically bind to the toxin. The profile of native membrane-bound α-glucosidase from An. gambiae larvae revealed a molecule that has the same molecular weight as the receptor Cqm1. In vitro data showed that An. gambiae agm1 gene codes an α-glucosidase that binds to Bin toxin, an requirement to be the receptor, and that larvae is likely to express the native Agm1 maltase, as a membrane-bound protein.

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Poster / Bacteria. B-22

Mutagenic analysis of loops in the receptor binding domain of Bacillus thuringiensis cry11Ba toxin

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Since no crystal structure of the Bacillus thuringiensis Cry11Ba toxin is currently available, the secondary-structure prediction for this toxin was threaded against the whole backbone database with Threader. A model was built that corresponded to the top hit Cry2Aa and Cry11Aa. Further, since receptor binding is a key determinant for the specificity of three-domain Cry toxins, analysis of the four putative surface loops (loop α-8, loop 1, loop 2, and loop 3) in domain II of the Cry11Ba toxin was been carried out to assess their role in receptor binding and toxicity. Site-directed mutagenesis of the cry11Ba gene was performed by converting the loop region amino acid residues into triple alanine mutations. Sixteen mutants were confirmed by sequenced and ligated into the pHT315 shuttle plasmid. The mutant plasmids were then transformed and expressed in B. thuringiensis strain 4D11. Data will be presented to show that alanine replacements of the loop regions of domain II resulted in altered toxicity to different mosquito species

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Poster / Bacteria. B-23

Use of Green Fluorescent Protein to monitor the pathology of a unique Yersinia sp,. able to kill many insect species within 72 hours.

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A new member of the Enterobacteriaceae has been isolated from a diseased grass grub Costelytra zealandica, Coleoptera: Scarabaeidae), field collected from New Zealand soils. The bacterium has broad host range, killing a range of insect species notably members of the Coleoptera and Lepidoptera within 72 hours post infection. Through the use of a Green Fluorescent Protein labelled derivative of the Enterobacteria, and using grass grub as a model system we show that at the initial stage of infection few bacteria are detected in the gut. Instead the bacteria invades the haemocoel where they rapidly multiply and destroy the haemocytes, there after the bacteria occupy every observable area of the insect system. This study defines the novel species of Enterobacteriaceae as a finely tuned pathogen able to invade and destroy the insect immune system before overwhelming the insect.

Poster / Bacteria. B-24

Phylogeny and host range testing of a a novel entomopathogenic species of Enterobacteriaceae,

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During routine sampling of larvae for use in bioassays, larvae from a particular area were often found to die quickly of a bacterial septicaemia. The larvae were set aside and screened for the presence of a bacterial pathogen. Healthy larvae were refed with the isolated bacteria and the symptoms were repeated. The larvae turned to an amber colouration within 4 -16 hours of ingestion, followed by a progressive browning leading to a blackened septicaemic state and mortality within 24-72 hours of infection. Phylogenetic analysis in conjunction with DNA-DNA hybridisation studies demonstrate that this is a new species of bacteria falling within the Genus Yersinia. The bacterium was found to have broad spectrum activity affecting insects of the families Coleoptera and Lepidoptera amongst others. Phlyogentic data is presented in conjunction with insect species affected and not affected by the new species.

Poster / Bacteria. B-25

Identification of three Zwittermicin A biosynthesis-related genes from Bacillus thuringiensis subsp. kurstaki strain YBT-1520

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Zwittermicin A (ZwA) is a novel, broad-spectrum linear aminopolyol antibiotic produced by some Bacillus cereus and Bacillus thuringiensis. However, only part of its biosynthesis cluster has been identified and characterized from B. cereus UW85. To better understand the biosynthesis cluster of Zwittermicin A, a bacterial artificial chromosome (BAC) library of Bacillus thuringiensis subsp. kurstaki strain YBT-1520, a ZwA-producing strain, was constructed. Two BAC clones, 1F8 and 5E2, were obtained by PCR, which overlap the known Zwittermicin A biosynthesis cluster of B. cereus UW85. This ZwA biosynthesis cluster is at least 38.6kb and is located on the chromosome, instead of the plasmid. Partial DNA sequencing revealed both BAC clones carry three new ZwA biosynthesis-related genes, zwa6, zwa5A and zwa5B, which were found at the corresponding location of B. cereus UW85. Putative amino acid sequences of these genes shown that ZWA6 is homologous to a typical carbamoyltransferase from Streptomyces avermitilis, while ZWA5A and ZWA5B are homologs of cysteine synthetase and ornithine cyclodeaminase which jointly synthesize 2, 3-diaminopropionate in the Viomycin biosynthesis pathway, respectively. The identification of these three genes further supports the hypothesized ZwA biosynthesis pathway.

Poster / Bacteria. B-26

A 106-kDa aminopeptidase is a putative receptor for Bacillus thuringiensis Cry11Ba toxin in the mosquito Anopheles gambiae

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Bacillus thuringiensis (Bt) insecticidal toxins bind to receptors on midgut epithelial cells of susceptible insects, and binding triggers biochemical events that lead to insect mortality. Recently, a 100-kDa aminopeptidase N (APN) was isolated from the brush border membrane (BBM) of Anopheles quadrimaculatus and shown to bind Cry11Ba toxin (Abdullah et al. 2006 BMC Biochem. 7:16). In our study, a 106-kDa APN released by phosphatidylinositol-specific phospholipase C (PI-PLC) from Anopheles gambiae BBM bound Cry11Ba toxin. Its corresponding cDNA was cloned. Analysis of the primary structure of this APN revealed a zinc-binding motif (HEIAH), three potential N-glycosylation sites and a predicted glycosylphosphatidyl- inositol (GPI) anchor site. Immunohistochemistry localized the APN to the microvilli of the posterior midgut. Furthermore, a 70-kDa fragment of the 106-kDa APN expressed in Escherichia coli bound Cry11Ba and reduced Cry11Ba toxicity to A. gambiae larvae. These data are evidence that the 106-kDa GPI-anchored APN is a specific binding protein, and probably a receptor, for Bt Cry11Ba toxin.

Poster / Bacteria. B-27

Identification of Aedes aegypti alkaline phosphatase receptors of Bacillus thuringiensis subsp. israelensis Cry 11A toxin

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A 65 kDa glycosyphosphatidyl-inositol (GPI)- anchored alkaline phosphatase (ALP) was reported as a functional receptor of Bacillus thuringiensis subsp. israelensis (Bti) Cry11A toxin in Aedes aegypti midgut (Fernandez et al., Biochem. J. (2006) 394:77–84) but the corresponding protein is still unknown. Three ALP transcripts encoding proteins ALP 1, ALP 2 and ALP 3 were cloned by A. aegypti midgut cDNA library construction and RT-PCR. Two of the proteins ALP 1 and ALP 2 were predicted as GPI-anchored proteins and were expressed in Escherichia coli. Ligand binding experiments showed that the biotin-labeled Cry11A toxin bound purified ALP 1 and ALP 2 from E. coli, and that non-labeled Cry11A toxin competed with the biotin-labeled toxin binding to ALP 1. Immunohistochemistry experiments in fourth instar A. aegypti larvae were performed by using anti-ALP 1 antibody. The data showed the ALP 1 protein is located in apical membrane of proximal and distal gastric caecae and of posterior midgut cells.

Poster / Bacteria. B-28

Identification and analysis of Clostridium bifermentans mosquitocidal proteins

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Clostridium bifermentans serovar malaysia is shown to be highly toxic to anopheline mosquito larvae, however the underlying toxin has not been identified. To identify these toxins we monitored the mosquitocidal activity in cultures during bacterial incubation. As shown earlier (Charles et al., Res. Microbiol. (1990) 141: 721-733) mosquitocidal activity is observed during the sporulation stage, with high activity observed by 8 hours. Charles et al also showed sporangium lysis occurs at about 12-13 hours. To identify the toxins we monitored toxicity in supernatants of bacterial cultures. As expected, at 8 hours, we observed high levels of toxicity in the pellet obtained following centrifugation of the bacterial culture. No toxicity was observed in the supernatant, showing no toxins are secreted. At 24 hours high level of toxicity is observed in supernatant fraction, as well as the pellet fraction. At five days no toxicity is observed in the supernatant fraction. Proteins in one-day culture were then compared to five-day supernatants, and bands that showed differential expression were analyzed using mass spectrophotometry. One of the proteins identified in one-day cultures was a putative pore-forming toxin, which differed from previously identified proteins from this strain. Attempts will be made to determine the mosquitocidal toxicity of this putative toxin.

Poster / Bacteria. B-29

Identification of a cadherin in Anopheles gambiae larvae as a putative receptor for Bacillus thuringiensis israelensis Cry4Ba toxin.

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Bacillus thuringiensis israelensis (Bti) is highly toxic to larvae of several mosquito species, including Anopheles gambiae. Since cadherins in lepidopteran larvae function as Bt toxin receptors, we reasoned that homologous proteins may have a similar function in mosquitoes. A protein with similarity to lepidopteran cadherins was identified in A. gambiae databases and the corresponding cadherin cDNA was cloned. The cDNA encodes a 195.3-kDa protein with a predicted leader peptide, 11 cadherin repeats, a membrane-proximal extracellular domain, a membrane spanning region and an internal cytoplasmic domain. Anti-serum prepared against E. coli-expressed cadherin, detected a 210-kDa protein in brush border membrane preparations. The cadherin-like protein, as visualized by immunohistochemistry of sectioned gut material, was localized in the posterior midgut on the apical portion of the brush border. Bti toxins were examined for their ability to bind A. gambiae cadherin. Cry4Ba toxin bound 210-kDa cadherin on blots of larval brush border protein. Rhodamine-labeled Cry4Ba toxin co-localized with cadherin on the microvilli of sectioned midgut tissue. Under non-denaturing conditions, Cry4Ba toxin bound cadherin expressed in Drosophila-S2 cells and binding was specific and competitive. These data identify this A. gambiae cadherin protein as a putative receptor for Cry4Ba toxin.

Poster / Bacteria. B-30

A proteomic approach to the identification of Cry4Ba binding proteins in midgut membranes from Aedes aegypti

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Cry 4Ba derived from the bacterium Bacillus thuringiensis israelensis is highly toxic to Aedes aegypti larvae. Cry toxin receptors have been extensively characterized in Lepidoptera. In mosquitoes, only alkaline phosphatase (ALP) is identified as a receptor for Cry11Aa toxin (Fernandez et al. 2006) and an aminopeptidase (APN) as a Cry11Ba binding protein and candidate receptor (Abdullah et al. 2006). A proteomic approach was taken to identify Cry4Ba binding proteins in brush border membrane vesicles (BBMV) prepared from A. aegypti larvae. BBMV proteins were separated by two-dimensional gel electrophoresis (2DE) followed by staining or blotting to membrane filters. Blots were probed with 125I-Cry4Ba. Toxin-binding protein spots were identified by MALDI-TOF mass spectrometry coupled to peptide mass fingerprinting (PMF) and database searching. Twelve Cry4Ba-binding proteins were identified in A. aegypti BBMV; including several alkaline phosphatases, actin and ATPase subunits. Alkaline phosphatase identifications were confirmed by western blotting. Several additional toxin binding proteins were identified by mass spectrometry and de novo sequencing, while several toxin-binding spots remain unidentified. Although there are limitations to this proteomics-based approach, the results provided an survey of Cry4Ba binding proteins in the A. aegypti larval midgut.

Poster / Bacteria. B-31

Sequence diversity of the Bacillus thuringiensis flagellin (H-antigen, Hag) protein - Comparison with H-serotype diversity

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In the early 1960's, H-serotyping, the immunological reaction to the bacterial flagellar antigen, flagellin, was developed as a classification tool for the Bacillus thuringiensis strains. We set to analyze the sequence diversity of the B. thuringiensis flagellin (H-antigen, Hag) protein and compare it with the H-serotype diversity. Some other B. cereus sensu lato species and strains were included for additional comparisons. The flagellin (hag) genes from 80 B. thuringiensis strains were amplified, cloned, their nucleotide sequences determined and translated in amino acids. A multiple sequence alignment revealed that the first 111 and the last 66 amino acids were conserved. They were referred to as the C1 and C2 regions, respectively. The central region, however, was highly variable and referred to as the V region. A bootstrapped neighbor-joining tree was generated, phylogenetic relationships were revealed, and correlations between

flagellin amino acid sequences and H-serotypes were shown. Next, flagellin sequence diversity was studied at the intra-H3-serotype level. Short specific amino acid sequences are revealed for specific H3 antigens.

Poster / Bacteria. B-32

Unusual organization associated to a tandem of IS231 may yield two peculiar cloverleaf secondary structures

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A 5.7-kb EcoRI fragment was cloned from plasmid DNA of Bacillus thuringiensis strain M15. It contains two insertion sequences (IS), IS231M2 and -M1 in the 5'-3' order, arranged in tandem, in same orientation, separated by a 540-bp region. The primary structure is typical of a composite transposon, here of 3847 bp in length, for which the name Tn231M is proposed. Each IS is delimited by 18-bp inverted repeats (IR), and flanked by 11-bp direct repeats (DR). Both IS share 99.3 % nucleotide identities. IS231M1 has a single open reading frame (ORF) which encodes a putative 477-amino-acid transposase. IS231M2 has two smaller ORFs: ORF1 and ORF2, which could code for polypeptides of 329 and 118 amino acids in length, respectively. Further analysis reveals that the regions upstream of IS231M2, and downstream of -M1, and the 540-bp region, contain additional pairs of IR and DR. Interestingly, potential annealing between all pairs of IR and DR could generate two unusual cloverleaf secondary structures.

Whether the putative composite transposon presented here, Tn231M, is capable of transposition is unknown. Likewise, whether any of the two unusual cloverlaf secondary structures might actually form in the bacterium and what their biological significance might be is also unknown. However, the unusual organization presented here is sufficient to warrant further investigation.

Poster / Bacteria. B-33

Lipid specificity of the Cyt1A/membrane interaction

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Understanding the molecular details of how the Bt toxin changes conformation in the presence of lipid membrane is important for elucidating the toxin's mode of action. Our previous binding studies with the toxin Cyt1A were performed using membranes made of chemically undefined lipid egg phosphatidyl choline (PC). Here we studied lipid specificity of Cyt1A binding regarding saturation of fatty acyl chain and chemical nature of the lipid head-group. Fluorescence of tryptophan was used as a measure of binding. The data were fitted with a single hyperbola, yielding a single value of apparent binding constant Kapp. Results show that Cyt1A binds to the membranes made of the saturated lipid 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) to a similar extent as to those made of the egg PC lipid mixture. Furthermore, the binding parameters were the same regardless of the fluidity of the DMPC membrane. This suggests that Cyt1A has no preference for binding to vesicles composed of either saturated or unsaturated lipids or to membranes in either liquid-crystal or gel state.

Poster / Bacteria. B-34

Mega assemblage of a mammalian cell-targeting and pore-forming toxin parasporin-2 from Bacillus thuringiensis

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Most of the pore-forming toxins (PFTs) from some pathogenic bacteria oligomerize during these cytotoxic actions and the oligomers are inserted into the membrane to permeabilize the membrane. Parasporin-2, anti-tumor crystal toxin, identified from Bacillus thuringiensis A1547 strain belongs to PFTs. Parasporin-2 monomers bind to lipid rafts of the target cell surface through GPI-anchored proteins, and then they form SDS-resistant oligomer inserted into the membrane. Although the oligomer seems to be important for the cytotoxicity and the pore-formation, the character and structure of oligomer are little understood. To investigate the native state of oligomer, we analyzed the oligomer under the non-denaturing condition. Using Blue-Native PAGE (BN-PAGE) analysis, parasporin-2 and structurally related oligomerizing-toxins, aerolysin and ε-toxin, formed much larger complexes than those size estimated in SDS-PAGE. To reveal the component of the huge complex, parasporin-2 tagged with N-terminal hexahistidine was oligomerized in human hepatocyte cancer (HepG2) cells and the oligomer was purified using nickel-affinity column. The purified proteins kept the mega assemblage on BN-PAGE and contained mainly the SDS-resistant parasporin-2 oligomer with minor cellar proteins. Thus, some PFTs form the supramolecular complex in plasma membrane of target cells during the cytotoxic action and the complex may be mainly constituted by SDS-resistant oligomers.

Poster / Bacteria. B-35

Functional genomics of Photorhabdus asymbiotica. Rapid Virulence Annotation (RVA) of pathogen genomes using invertebrate models.

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Photorhabdus asymbiotica is a symbiont of entomopathogenic nematodes that is emerging as a human pathogen. The genome sequence is almost completed and the annotation is underway. We are interested in the functional annotation of virulence factors and as such have developed a screening method using three model organisms: Manduca sexta, Caenorhabditis elegans and Acanthamoeba polyphaga. Screening a genomic library of P. asymbiotica with these three organisms has allowed the identification of toxins, secondary metabolites, secretion systems and completely novel virulence factors. Mapping these regions in the genome and comparing with P. luminescens TT01 (an insect-only pathogen sequenced strain) has provided clues about virulence regions that are exclusive to the human pathogen and play a role in infection. This Rapid Virulence Annotation (RVA) is also a powerful tool to identify virulence genes in other sequenced bacterial pathogens, useful in bridging the knowledge gap in the the post-genomic era. To illustrate the general utility of this approach we also present the preliminary results of the application of RVA technology to a plant pathogen, Pseudomonas syringae. Finally in addition to providing an alternative to mammalian testing RVA identifies of new targets for the development of novel insecticides, nematicides and anti-protist drugs.

Poster / Bacteria. B-36

Susceptibility of Spodoptera exigua to 9 toxins from Bacillus thuringiensis

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The beet armyworm Spodoptera exigua (Hübner) is a polyphagous insect pest that is widely distributed and causes serious damage to numerous cultivated crops, such us tomatoes, cabbage, pepper and

cotton. An optimal control of insect pest populations requires information on differences in insecticide susceptibility (dose-mortality and sublethal effects). In the present work nine Cry toxins from B. thuringiensis have been tested for their activity against three laboratory strains of S. exigua original from France, Holland and Spain. Cry1Ca, Cry1Da and Cry1Fa were the most effective toxins in mortality assays, whereas Cry1Aa and Cry1Ac were marginally toxic and the rest of toxins were non toxic. Interestingly, the effect of these Cry toxins on growth inhibition followed a different pattern: Cry1Aa, Cry1Ab, Cry1Ac, Cry1Fa and Cry2Aa produced significant larval growth inhibition, whereas Cry1Ca and Cry1Da did not significantly affect larval growth. Significant differences in susceptibility among strains were found for Cry1Ab and Cry1Da.The use of B. thuringiensis formulations to control S. exigua has to take into account the variability of populations regarding their susceptibility (mortality and other sublethal effects) to the different Cry toxins.

Poster / Bacteria. B-37

The metabolic regulation of thuringiensin biosynthesis by Bacillus thuringiensis strain YBT-1532

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The thuringiensin is a kind of adenine derivative produced by some strains of Bacillus thuringiensis. It has showed the broad-spectrum insecticidal activity against insects, mites and nematodes, such as squama. The biosynthesis and metabolic regulation of thuringiensin production were explored in the Bacillus thuringiensis YBT-1532 in this paper. In the semisynthetic media, thuringiensin yield increased from 67.8mg/g to 75.6~93.6mg/g by separatery addition of eight amino acids such as asparagine and glutamine, which serve as the precursors of de novo pathway of adenine, and increased from 67.8mg/g to 83.5~93.5mg/g by separatery addition of four organic acids which could facilitate the synthesis of above amino acid. In a continuous culture system of Bacillus thuringiensis YBT-1532 (D=0.12 h-1), pyruvate content and the ability to synthesize PHB were decreased with 0.3 g/L citrate addition, the activities of pyruvate kinase and glucose-6-phosphate dehydrogenase were 29.0% lower and 42.1%higher than those of the control, respectively. Thuringiensin synthesis yield increased by 14.7% when compared with control. The results above demonstrated that the citrate addition attenuated glycolytic flux, and increased the carbon metabolic flux in the pentose phosphate pathway, respectively. The changes were obviously in favor of more substrates supplying for thuringiensin synthesis and less for pyruvate production, which consequently decreased the PHB production and then increased the thuringiensin synthesis level. The yields of adenine and thuringiensin were increased significantly with addition of 1.0g/L formate in resting cell of B. thuringiensis YBT-1532, NADH concentration increased by 19.6%, intracellular enzymes activities of formate dehydrogenase, pyruvate kinase and glucose-6-phosphate dehydrogenase enhanced by 2-fold, 4.25-fold and 2.5-fold when compared with control, respectively. Intracellular production of aspartate, pyruvate, citrate and adenine were 65.0%, 75.0%, 31.9% and 71.4% higher than those of the control, respectively, and thuringiensin yield increased by 90%. Appropriate addition of DMSO (10 mL/L), SDS (0.10 g/L), penicillin (300 U/mL, 9 h) and fosformycin (50 U/mL, 9 h) facilitated thuringiensin synthesis evidently, respectively, in the batch culture. At the case of penicillin addition, intracellular thuringiensin and activity of intracellular phosphatase decreased by 12.0% and 15.3%, respectively. Intracellular dephosphorylated thuringiensin and thuringiensin secretion enhanced by 25.0% and 71.8% compared to control. Penicillin addition enhanced cell permeability and facilitated thuringiensin excretion.

Key words: Bacillus thuringiensis; thuringiensin; biosynthesis and metabolic regulation; penicillin;

Subject: National Natural Science Fund 30400003

MICROBIAL CONTROL

Poster / Microbial Control. MC-01

Development of a repository for baculoviruses (a Baculobank) in Canada

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Forests are constantly at risk from fires, pathogens and insect pests. The use of environmentally acceptable alternatives to chemical pesticides continues to play a role in the establishment of a sustainable forest industry and provides a compelling case for accelerated discovery and commercialization of forest biopesticide technologies. Of the 15 different virus families that infect insects, the Baculoviridae are the most attractive because they are restricted to insects, are host specific, are known to cause epizootics and are occluded in a stable form that is easy to produce and purify for microbial control. Application of baculoviruses to outbreak populations of forest insect pests can suppress these populations before significant damage occurs to the forest.

To date, four different nucleopolyhedroviruses have been developed and registered for use as microbial control agents in forestry in Canada. Unfortunately, stocks of specific baculoviruses, required to control many forest insect pests, are not readily available for scale-up production, let alone for control applications. The creation of a repository for baculoviruses (a Baculobank) will establish a "pipeline" for the development of baculovirus technologies. With the establishment of the Baculobank, new baculoviruses will be identified, stored, registered, produced, commercialized and made available to forest pest management stakeholders.

Poster / Microbial Control. MC-02

Susceptibility of Cydia pomonella to mixed preparation of granulosis virus and Bacillus thuringiensis

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Researches on biological control with granulosis viruses were carried out in the Institute of Biology, University of Latvia, Laboratory of Experimental Entomology since 1986. The aim of the present study was to investigate the susceptibility of the codling moth Cydia pomonella (L.) (Lepidoptera: Torticidae) to mixed preparation of Cydia pomonella granulosis virus (CpGV) and Bacillus thuringiensis var. kurstaki. Granulosis virus isolated from the apple codling moth by the activation of latent infection and Bacillus thuringiensis var. kurstaki (cry I) obtained from culture collection were used. Larva of Cydia pomonella reared in laboratory on semi-synthetic media was used in experiments. Different amount of GV and B. thuringiensis are added to nutrient media. Mode of action of mixed preparation was evaluated. Efficiency of preparation was expressed as the percentage mortality. Presence of CpGV or B. thuringiensis in dead larvae was checked by phase contrast and dark field microscope. The results of bioassay demonstrated that LT 50 of mixed preparation was 3 to 5 days. This work has been financially supported by the grants from the Latvian Council of Sciences.

Poster / Microbial Control. MC-03

Semiochemical autodissemination of tortricid viruses in the orchard

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An alternate strategy to that of spray application for the delivery of two totricid viruses was assessed in the orchard, using attract and contaminate devices. The Adoxophyes orana (Ador) sex pheromone was used in the lure for the A. orana granulovirus (AdorGV) dispensers and either the Cydia pomonella (Cp) sex pheromone or a bisexual attractant (pear kairomone (Ethyl (2E, 4Z)-2,4-decadienoate, DA2313) or a combination of both were used in the lures for the CpGV dispensers. Virus transfer experiments were conducted in the orchard in year 1 and year 2, to compare the spread of infectious CpGV and AdorGV in the orchard, using powder and liquid formulations of the viruses, respectively. In the third year, we carried out a large scale orchard experiment involving twelve one hectare plots (triplicate plots for four treatments), in a large commercial cider orchard focusing only on codling moth. Sting damage and deep entry damage were assessed on three occasions during the season. Samples of stings from damaged apples, larvae from deep entry damage and overwintering larvae were checked for the presence of CpGV, using insect based bioassays and molecular techniques.

Poster / Microbial Control. MC-04

Antifeeding toxin or just bad taste?

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With the a current trend towards lessening the environmental impact of pest control, and a continuing desire to mitigate losses incurred from insect borne diseases and insect mediated crop damage, the search for new biological insecticidal toxins is ongoing. The Toxin Complex (Tc) family of proteins are a relatively new class of insecticidal proteins showing promise for use in insect control programs. Tc toxins are prevalent among nematode associated bacteria (e.g. Photorhabdus luminescens) and Enterobacteriaceae associated with soil environments (e.g. Serratia entomophila). We have examined the anti-feeding and pathogenic effects of a range of Tc containing bacteria against the New Zealand grass grub, Costelytra zealandica (White), and the diamond back moth, Plutella xylostella (L.). The results indicate a range of feeding responses and pathological effects from the Tc containing bacteria. A discussion of the differences in efficacy will be presented with emphasis on the feeding response.

Poster / Microbial Control. MC-05

Proteinase activities and proteolytical processing of the B.t.-corn-toxin Cry3Bb1 in the midgut of Western Corn Rootworm (Diabrotica virgifera virgifera)

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The Western Corn Rootworm is an economical important pest in corn (Zea mays L.). One possibility for its control is the cultivation of transgenic corn expressing the Bacillus thuringiensis toxin Cry3Bb1. However, cultivation of B.t.-corn may result in resistant pest populations.

The potential of insect resistance to B.t.-toxins can be located at any step of the toxic pathway: ingestion, solubilization, proteolytic processing, binding to specific receptors, membrane integration, pore formation, cell lysis, and insect death. However, in other B.t.-toxin-pest-systems, resistance is mainly proteinase- or receptor-mediated. To establish reference systems for the characterization of potential available resistant individuals, studies on proteinase activities and proteolytical processing of the B.t.-corn-toxin Cry3Bb1 were carried out with midgut fluid of susceptible WCR 3rd instar larvae (pH 5,75).

As a result, the digestive serin-endopeptidases trypsin, chymotrypsin, and elastase as well as aminopeptidase – an exopeptidase - were identified. Due to the acid midgut fluid, in Chrysomelidae cysteine-endopeptidases were expected. Accordingly, high activities of Cathepsin L, Cathepsin B, and Cathepsin H were found.

IOBC wprs Bulletin (Meeting: IOBC wprs Study Group "Ecological Impact of Genetically Modified Organisms", November 26-29, 2003, PraguBy in vitro incubation with WCR midgut fluid, the B.t.-corn-toxin Cry3Bb1 was processed. To identify the midgut proteinases, which are responsible for the proteolytical processing, available model proteinases were used to simulate the midgut conditions.

Poster / Microbial Control. MC-06

A peptide derived from Manduca sexta Bt-R1a cadherin enhances activity of commercial Bt formulation on Bt-susceptible and Bt-resistant insects

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The cadherin Bt-R1a is a receptor for Bacillus thuringiensis (Bt) Cry1A toxins in midgut epithelia of tobacco hornworm (Manduca sexta). The Bt-R1a region most proximal to the cell membrane (CR12-MPED) is the essential binding region required for Cry1Ab-mediated cytotoxicity. Previously, we discovered that a peptide containing this region expressed in Escherichia coli functions as an enhancer of Cry1A toxicity against lepidopteran larvae. We now demonstrate that a derivative of the enhancer peptide increases the activity of a commercial Bt sprayable product in plant-based bioassays. The enhancer peptide (called BtBoosterTM) is being developed by InsectiGen, Inc. as a commercial product that enhances the performance of Bt products. BtBoosterTM enhanced Javelin® WG (CertisUSA) (contains the Bt NRD12 strain) in tomato excised-leaf bioassays against Helicoverpa zea. Additional excised-leaf bioassays using soybean and cabbage consistently demonstrated that BtBoosterTM significantly enhanced Javelin® WG. We also demonstrated that BtBoosterTM was able to partially overcome resistance in a diamondback moth, Plutella xylostella, strain that had developed resistance to Bt in the field. Bioassays with Javelin® WG plus BtBoosterTM against resistant P. xylostella larvae consistently showed that the addition of BtBoosterTM to the biopesticide significantly enhanced mortality in both excised-leaf and whole plant greenhouse experiments.

Poster / Microbial Control. MC-07

Efficacy of Beauveria bassiana (Bals.) Vuill. against the tarnished plant bug, Lygus lineolaris L., in strawberry crop

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The entomopathogenic fungus Beauveria bassiana has a high insecticide potential to control the populations of the tarnished plant bug, Lygus lineolaris, a significant pest of strawberry crop. Results of screening experiment shown that L. lineolaris adults were susceptible to several B. bassiana isolates. A second screening test with Coleomegilla maculata, a natural enemy found in strawberry crop, was also performed in order to select the isolate which have lower entomopathegenic impact on this insect. Based on results obtained on both insect species and on the ecozone origin of the B. bassiana isolates, INRS-IP and INRS-CFL isolates were selected for further

experiments. The LC50 values of these two isolates against L. lineolaris adults were, respectively, of 7.8x105 and 5.3x105 conidia/ml, and AST values of 4.54 and 4.41 days at a concentration of 1x108 conidia/ml. Results also indicated that L. lineolaris nymphs are more susceptible than adults to the selected isolates. During field experiments, using a randomized block design with four replicates, two rates (1x1011 and 1x1013 conidia/ha) of INRS-IP and INRS-CFL isolates were applied weekly during four weeks. These multiple applications triggered a significant reduction of L. lineolaris nymph populations in strawberry crop. Twenty-seven days after the first application, a significant difference was observed between the mean population densities of nymph survival in all B. bassiana-treated plots (less than 1 insect/ 5 plants) compared to control plots (4 insects/ 5 plants). During the field experiment, persistence of insecticide activity and viability of B. bassiana conidia were also monitored. The results showed the presence of viable and infective conidia up to 6 days after each application on strawberry foliage. Moreover, the multiple applications of B. bassiana at the rate of 1x1013 conidia/ha triggered a significant reduction of strawberry fruit injuries induced by L. lineolaris feeding behavior compared to the control plots.

Poster / Microbial Control. MC-08

Effect of soil management on naturally occurring entomopathogenic fungi during the transition to an organic farming system

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We examined the interaction between soil disturbance and initial cover crop type on weed populations, soil quality, and economic performance during the three-year transition from conventional to organic production in a feed grain rotation in central Pennsylvania. Our experiment included four production systems comprised of a factorial combination of two levels of primary tillage (full vs. minimum) and two types of initial cover crop (perennial sod/legume vs. annual cereal grain/legume). We assayed soil for entomopathogenic fungi (EPF) using Galleria mellonella four times during each field season in each treatment and examined the relationship between EPF and biotic and abiotic soil characteristics. We detected three species of EPF: Metarhizium anisopliae, Beauvaria bassiana, and Paecilomyces fumosoroseus. EPF were favored in systems that minimized tillage and utilized a sod-forming timothy/red clover cover crop mixture. Greater detection of EPF occurred during the initial cover crop than during subsequuent soybean or maize production. The presence of EPF was positively associated with soil moisture and the concentration of permanganate oxidizable carbon in soil. This study can be used to inform farmers of the impacts of management practices on soil function, specifically conservation biological control.

Poster / Microbial Control. MC-09

Pathogenicity of Metarhizium anisopliae expressing the scorpion toxin (AaIT) against coffee berry borer, Hypothenemus hampei (Coleoptera:Curculionidae)

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Coffee berry borer (CBB) is the Worlds most devastating coffee pest causing an estimated of US$500 million annually in losses and control costs. Beauveria bassiana and Metarhizium anisopliae have been employed to control this pest but their slow kill is an important factor constraining their use. M. anisopliae has been modified to express the scorpion toxin (AaIT) in insect haemolymph and this dramatically increased pathogenicity against Manduca sexta and Aedes aegypti. Here, we demonstrate that the recombinant M. anisopliae strain expressing AaIT (MaPcTox) was also dramatically more virulent to CBB. We evaluated several spore concentrations (1x101 through 1x107spores/ml) of both wild type and recombinant strains. Results demonstrated that at concentrations of 1x102 and 1x103 spores/ml, the recombinant strain significantly increased mortality of CBB by 40% and 55%, respectively. The medial lethal concentration (LC50) was reduced by 18 fold and the medial lethal time (LT50) was reduced by 21.5% at the highest concentration evaluated. The LT50 was reached in 2.85 ± 0.08 days, a significant finding since it is the first occasion that an entomopathogenic fungus killed the CBB in less than 3 days. In future research we will insert the AaIT gene in B. bassiana and compare its efficacy against CBB.

Poster / Microbial Control. MC-10

Effect of amber disease on regulation of midgut proteases in the New Zealand grass grub Costelytra zealandica

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The endemic grass grub, Costelytra zealandica, is an important pasture pest in New Zealand. The bacteria Serratia entomophila and S. proteamaculans cause amber disease in C. zealandica larvae. Strains of S. entomophila have been used to successfully control C. zealandica in the field. Symptoms of amber disease in C. zealandica larvae include: cessation of feeding; rapid clearance of midgut contents and a near elimination of proteolytic activity associated with the midgut; amber colouration of the midgut; and eventual death of larvae. Although the bacterial genes contributing to amber disease are now known, the mode of action of bacterial toxins during amber disease remains largely unknown. While proteolytic enzyme titre in the midgut drops to very low levels following infection, results from quantitative PCR (qPCR) experiments on midgut epithelial cells have demonstrated that serine protease genes are not down regulated following infection. Proteomic analysis reveals an increase in protein accumulation in gut cells from amber diseased C. zealandica. These results suggest that disease effects enzyme formation and transport rather than transcription. While histology shows no obvious gross cytotoxic effects on the midgut cells, intracellular phenotypes are currently under investigation. A model for the mechanism of amber disease mode of action in C. zealandica will be discussed in light of our recent results.

Poster / Microbial Control. MC-11

Susceptibility of Rhagoletis indifferens (Diptera: Tephritidae) larvae and pupae to infection by Beauveria bassiana

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In the Pacific Northwest of North America, western cherry fruit fly, Rhagoletis indifferens (Diptera: Tephritidae) drop from infested fruit as late instar larvae and overwinter as pupae in the orchard soil. The objective of this study was to determine the susceptibility of these two soil residing stages of R. indifferens to infection by an orchard isolate of Beauveria bassiana. In sand-based laboratory bioassays, last instar R. indifferens larvae were found to be susceptible to infection by the B. bassiana conidia. Larvae were occasionally killed when exposed to the higher conidial concentrations, however, more frequently, the infected larvae would pupate and the pupae would show signs of mycosis within seven days resulting in significant ($P < 0.05$) fruit fly mortality. A significant ($P < 0.05$) proportion of the R. indifferens pupae that were exposed to B. bassiana treated sand within 12 hours of pupation were susceptible to fungal infection. Exposure of pupae to the fungus more than 24 hours after pupation did not result in statistically significant mortality, when compared with the control. These results suggest a short potential window of 24 to 48 h for fly susceptibility within treated soil at these specific stages of development.

Poster / Microbial Control. MC-12

Quantitative expression of delta-endotoxin protein in HD-1-S-2005, the proposed new international reference standard for Bacillus thuringiensis

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Bacillus thuringiensis subsp. kurstaki strain HD-1 was adopted as the international reference standard for Bt by the Society for Invertebrate Pathology more than 35 years ago. Since then, the strain has been specially prepared as the standard from single production lots and assigned a potency value based on its activity against larvae of the cabbage looper, Trichoplusia ni. Samples, in powder form, have been made available to the research community at large through SIP. The current stock, "HD-1-S-1980," is now exhausted and a new production lot, manufactured and calibrated by Valent Biosciences, Inc., has been proposed as the new standard under the name "HD-1-S-2005." As part of the effort to fully characterize the new product, the amount of activated δ-endotoxin protein that it produces was measured relative to dry weight, total alkali-soluble protein and protease-resistant protein, and the yield per cell determined.

Poster / Microbial Control. MC-13

Bacillus thuringiensis international reference standard workshop

To Bt or not to Bt: do we need an international reference standard? Pros, cons and future directions

Workshop panel: Michael Brownbridge (moderator)1, Dirk Ave2, Gary Benzon3, Wendy Gelernter4, Larry Gringorten (convenor)5 1AgResearch Ltd., Agriculture and Science Centre, Lincoln, New Zealand, 2Valent Biosciences, Inc., Libertyville, IL, USA, 3Benzon Research, Carlisle, PA, USA, 4Pace Consulting, San Diego, CA, USA, 5Canadian Forest Service, Sault Ste. Marie, Ontario, Canada

A Bacillus thuringiensis powder manufactured by Valent Biosciences has been proposed as the next Bt international reference standard, to be distributed under the name HD1S2005. A workshop will be held under joint auspices of the Microbial Control and Bacterial Divisions to discuss the product and provide information about its properties, uses and distribution. A panel composed of industry representatives and researchers will provide background and technical information, and will facilitate discussion with the audience on issues ranging from the need for an international reference standard to the adoption of additional standards and the role the Society for Invertebrate Pathology should play.

MONDAY PM

VIRUSES I

Poster / Viruses. V-01

Commensal and mutualistic relationships of reoviruses with their parasitoid wasp hosts

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During evolution, certain endoparasitoid wasps have developed mechanisms to suppress the defence systems of their hosts. For this purpose, these species, all of which belong to the families Ichneumonidae and Braconidae, inject various kinds of virus-like particles. The most studied of these particles are classified as polydnaviruses (family Polydnaviridae). Over the past decade, it has also been shown that several wasp species harbour reoviruses (family Reoviridae), and that two of these suppress host defence system, allowing the development of the parasitoid eggs. In this paper, we summarize the key features of these viruses and their relationships with their wasp hosts. Five reoviruses are known that appear to be non-pathogenic for the wasps. Three of these, McRVLP, HeRV, OpRVLP, use their wasp hosts as vectors, and do not appear to be involved in host defence suppression. The fourth, DpRV-1, is a commensal reovirus detected in most field populations of the wasp, Diadromus pulchellus. This virus is always found associated with an ascovirus, DpAV-4a, which is indispensable for host immune suppression. Although DpRV-1 has not been shown to directly increase D. pulchellus parasitic success, it may contribute to this success by retarding DpAV-4a replication in the wasp. The fifth reovirus, DpRV-2, occurs in a specific population of D. pulchellus in which DpRV-1 and DpAV-4 are absent. This virus has a mutualistic relationship with its wasp host, as its injection by females during oviposition is essential for host immunosuppression. Interestingly, these viruses belong to several different reovirus genera.

Poster / Viruses. V-02

Evolution of ascovirus from baculovirus-a hypothesis

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Ascovirus, iridovirus, asfarvirus and poxvirus are all cytoplasmic DNA viruses and the evolutionary origin of cytoplasmic DNA viruses has never been well understood. Genetic and molecular data were used to test if the four cytoplasmic DNA virus families evolved from nuclear replicating baculovirus and how the four families were related. Molecular phylogenetic analyses using DNA polymerase with parsimony and likelihood programs predicted that cytoplasmic DNA viruses might have evolved from nuclear replicating baculoviruses and ascoviruses are more closely related to baculoviruses than the other three cytoplasmic DNA viruses. It was also predicted that ascovirus evolved into iridovirus which might have given rise to other cytoplasmic DNA virus such as poxvirus. A genome sequence comparison also indicated that ascovirus has more baculovirus protein homologues than does iridovirus. Spodoptera frugiperda ascovirus 1a (SfAV-1a), has 20 baculovirus protein homologues, whereas the iridovirus (Chilo iridescent virus) has only 5 baculovirus protein homologues. If evolution is a continuous process and closely related organisms share more morphological and structural similarities as well sharing more protein homologues, it is likely baculovirus diverged into ascovirus which diverged into iridovirus. The iridivirus might have given rise to the other cytoplasmic DNA viruses such as poxvirus.

Poster / Viruses. V-03

Is the presence of persistent baculovirus infections linked to insect immunity?

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Molecular detection techniques have revealed the high prevalence of persistent baculovirus infections in both laboratory-reared and wild populations of insects. However, the exact nature of these infections and the mechanisms by which they are established, maintained and re-activated are unknown; persistence could be intimately linked to host immunity. Invertebrate immunity is dependent on a complex innate immune system. Assays of haemolymph phenoloxidase and lysozyme, and encapsulation of abiotic filaments are limited as measures of immune capacity, but are frequently carried out as indicators of immunocompetence.

An assay for phenoloxidase activity was tested with the spruce budworm Choristoneura fumiferana. Activity was highly variable between individuals, and no sex-specific differences were detected. Standard lysozyme assays were unable to detect activity in naïve C. fumiferana, but immune stimulation with LPS induced measurable activity. Encapsulation of nylon fishing line inserted into C. fumiferana provides an additional measure of immune function. Automated image analysis techniques for quantifying the extent of cellular coverage and melanisation are under investigation.

Assays for commonly used indicators of host immunity have been optimised for C. fumiferana, and used to investigate interactions between immunocompetence, traditional measures of host fitness such as pupal weight, and also the presence of persistent baculovirus infections.

Poster / Viruses. V-04

Isolation and molecular characterization of a nucleopolyhedrovirus, granulovirus and cypovirus from populations of the western spruce budworm Choristoneura occidentalis (Lepidoptera: Tortricidae) in British Columbia, Canada.

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Geographically separate populations of western spruce budworm (Choristoneura occidentalis) were sampled in British Columbia to investigate the prevalence of viral pathogens within the natural system. A nucleopolyhedrovirus, designated ChocNPV, was characterized via restriction fragment length polymorphism (RFLP) and sequence analysis. The polyhedrin gene of ChcoNPV was fully sequenced, allowing the phylogenetic relationship between ChocNPV and other known baculovirus polyhedrin gene sequences to be established. Interestingly, spindles were observed in the host cell cytoplasm during infection with ChocNPV. The viral spindlin gene, believed to be responsible for the production of these structures, was fully sequenced. Sequence analysis and RFLP was used to characterize a granulovirus, ChocGV. Sequence data for the full granulin gene was found to be identical to the ChocGV granulin gene previously reported. A cypovirus, CoCPV, was also isolated. Sequence data was obtained for five of the ten RNA genome segments, including that of segment 10, the polyhedrin gene. Phylogenetic analysis aligned this virus with "type 16" viruses, most closely related to a cypovirus isolated from the eastern spruce budworm C. fumiferana. The high prevalence of pathogenic viruses shown in this study has interesting implications for the natural dynamics of C. occidentalis populations.

Poster / Viruses. V-05

Selection of a new virus isolate to control CpGV-resistant codling moth populations

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The Cydia pomonella granulovirus (CpGV) is being successfully applied worldwide not only in organic but also in IP orchards for the control of the codling moth (CM), Cydia pomonella. Resistance of local CM populations towards CpGV has recently been reported to occur after intensive sprayings over numerous years. In order to overcome this resistance, a new mixture of genotypes has been selected out from the original gene-pool of the commercialised CpGV-genotypes on a resistant CM population. For the first time, it was possible to develop a new product by selection of a new mixture of genotypes of CpGV, which is able to control resistant CM populations. Results from bioassays in the laboratory as well as from field trials are presented. The method of selecting new genotype mixtures of baculoviruses provides a powerful tool of developing new active compositions of genotypes that can overcome resistance in the future.

Poster / Viruses. V-06

Fast- and slow-killing genotypic variants in a Dutch isolate of Adoxophyes orana nucleopolyhedrovirus

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The smaller tea tortrix Adoxophyes honmai (Lepidoptera: tortricidae) is one of the most important pests of tea plants in Japan. Four different geographical strains of nucleopolyhedrovirus (NPV) that are infectious to A. honmai have been identified. Two of these were isolated from A. honmai in Tsukuba and Tokyo, Japan, and designated AdhoNPV-Ts and AdhoNPV-To, respectively. The other two, AdorNPV-En and AdorNPV-Ne, were isolated from A. orana in England and the Netherlands. The genomes of these four NPV strains have distinct restriction endonuclease fragment patterns. Bioassays in neonate A. honmai showed that AdorNPV-En is a typical fast-killing-type NPV, whereas both AdhoNPV-Ts and AdhoNPV-To are slow-killing NPVs. On the other hand, insects infected with AdorNPV-Ne died during all stages from neonate to final (fifth) instar, suggesting that wild-type AdorNPV-Ne is a mixture of fast- and slow-killing NPVs. Here, we investigated the genotypic variation in AdorNPV-Ne, and obtained isolates having two distinctive killing speeds using an in vivo cloning method. The difference in killing time of the two isolates was more than 10 days. Restriction endonuclease analysis showed that the two isolates have unique genotypes but share many co-migrating fragments, indicating that they are variants of the same species.

Poster / Viruses. V-07

Combined use of CpGV granulovirus and Trichogramma wasps against codling moth, an apple pest

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The project studies the use of CpGV granulovirus with Trichogramma minutum Riley (Hymenoptera: Trichogrammatidae) as part of a biological control program against Cydia pomonella L. (Lepidoptera: Tortricidae) in apple orchards. Various bioassays were conducted in a laboratory to measure the virulence of CpGV on C. pomonella neonates larvae and on newly laid eggs. Results indicated that the virus induced a higher mortality rate in larvae when applied on artificial diet rather than on the eggs. Furthermore, the mortality rate of the codling moth larvae varied directly with the concentration of the granulovirus suspension.
In another laboratory test, granulovirus was applied on C. pomonella eggs to determine the effect on the parasitism rate and on the emergence rate of T. minutum. The eggs were sprayed with three different granulovirus concentrations, an aqueous solution containing powdered milk and sugar, and a control consisting of distilled water. Results showed a significant difference between the control and the viral treatments in the T. minutum parasitism rate. However, no significant difference was observed in the number of T. minutum emerged per parasited egg.

Poster / Viruses. V-08

Recombinant Sindbis viruses that regulate apoptosis in the C6/36 Aedes albopictus cell line

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Sindbis virus (SINV), a mosquito-borne virus, belongs to the genus Alphavirus in the Togaviridae family. SINV induces dramatic apoptosis in most mammalian cell lines, but mosquito cell lines, such as C6/36 cells from Aedes albopictus, exhibit only moderate cytopathic effects with persistent SINV infection. In this study, we are using SINV as a vector to express either pro-apoptotic or anti-apoptotic genes in C6/36 cells, in order to study apoptosis regulation in mosquito cells. Expression of the pro-apoptotic genes Drosophila Reaper (Rpr) or Aedes aegypti Michelob_x (Mx) using SINV caused apoptosis in C6/36 cells. We observed chromosomal DNA condensation and fragmentation typical of apoptosis by Hoechst staining and DNA agarose gel electrophoresis after Rpr or Mx recombinant virus infection. We also detected cell membrane blebbing and caspase activation after infection. These apoptotic features were accompanied by high production of virus during the infection in mosquito cells. Expression of the baculovirus caspase inhibitor P35 inhibited actinomycin D-induced caspase activity in SINV-infected C6/36 cells. Based on the data above, these recombinant viruses can be used as tools to study apoptosis and its effects on vector competency in mosquitoes.

Poster / Viruses. V-09

Expression analysis of polydnavirus rep genes in Choristoneura fumiferana larvae parasitized by Tranosema rostrale

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Polydnaviruses (PDV) are dsDNA viruses with segmented genomes. They are obligate symbionts of some endoparasitic wasps belonging to the families Ichneumonidae and Braconidae. Viral replication occurs in calyx cells of the wasp ovary and viral particles accumulate in the oviducts, from which they are injected during oviposition into lepidopteran host larvae; there, viral genes are expressed, causing physiological dysfunctions that benefit the wasp larva. The genomes of ichneumonid PDVs (ichnoviruses or IV) contain a gene family (rep) whose members display no similarity to known non-PDV genes and no recognizable motifs; this is the most numerically important IV gene family. Seventeen putative rep ORFs were identified in the genome of the Tranosema rostrale ichnovirus (TrIV); 13 of these were detected by PCR amplification of cDNAs, in a cDNA library constructed from TrIV-injected Choristoneura fumiferana caterpillars. Temporal and tissue-specific q-PCR transcriptional analysis of those rep genes, in infected last-instar C. fumiferana larvae, will be presented.

Poster / Viruses. V-10

Campoletis sonorensis polydnavirus ankyrin and cys-motif genes affect on baculovirus replication in H. virescens and H. zea.

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The potential for Campoletis sonorensis ichnovirus (CsIV) cys-motif and viral ankyrin genes to synergize the Autographica californica baculovirus (AcMNPV) infections in permissive (H.v.) and semi-permissive (H.z.) hosts was examined by infecting Heliothis virescens and Helicoverpa zea larvae with recombinant baculovirus expressing CsIV genes. Larvae were infected by intra-hemoceolic injection of recombinant polyhedrin negative viruses and feeding of recombinant polyhedrin positive virus expressing a viral ankyrin genes or a cys-motif genes. These studies demonstrated that co-expression of polydnavirus genes in AcMNPV enhanced susceptibility infectivity of the virus. To further study the activity of these genes we are assessing the effects of CsIV genes on apoptosis in cell lines with and without virus infection. We have demonstrated that some ankyrin genes do interact with cellular apoptotic pathways.

Poster / Viruses. V-11

Identification and characterization of novel RNA sequences associated with late male-killing in the oriental tea tortrix, Homona magnanima (Lepidoptera: Tortricidae)

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A female-biased sex ratio was found in the oriental tea tortorix, Homona magnanima, in Tsukuba, Ibaraki, Japan. The cause of biased sex ratio is late male-killing, that is, only males die at larval or pupal stages. The causative agent is not either Wolbachia, Spiroplasma, Rickettsia and Flavobacteria reported as early male-killing (embryonic male death) agents or protozoa as late male-killing agents in mosquitoes. The causative agent of H. magnanima probably consists of a particle as large as small viruses. We partially purified an agent causing late male-killing by virus-purified method and confirmed male-killing agent in the purified fraction by bioassay. To identify late male-killing specific nucleic acids, we conducted RAPD analysis. Two RNA sequences, MK1081 and MK1240 were found specifically in female-biased H. magnanima but not in the

normal strain. A BLAST research revealed that their nucleotide and deduced amino acid sequences did not match any other known sequences. When the normal strain larvae were injected with purified agent, progenies showed female-biased sex ratio, exhibited both MK1081 and MK1240, but progenies showed normal sex ratio did not exhibit MK1081 or MK1240. These results indicated that MK1081 and MK1240 associated with late male-killing in H. magnanima.

Poster / Viruses. V-12

The role of innate immunity of gypsy moth under exogenous infection by nucleopolyhedrovirus.

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It is well known that insects have as defensive barriers prevented virus penetration in host organism as defensive mechanisms prevented virus development into the host. In our investigations we studied dependence between insect immune function and its mortality from virus inoculation. We have estimated innate immunity of fourth instar larvae (encapsulation rate, phenoloxidase (PhO) activity in lymph, and haemocytes amount). Then we exposed larvae to LD50 dose of nucleopolyhedrovirus and insect mortality from virus has been determined. We have found that insects which were resistant to virus inoculation have less PhO activity and fewer amounts of haemocytes in haemolymph. The encapsulation rate was same for all investigated insects. Possibly, the larvae with high PhO activity and high amount of haemocytes have low value of other parameters of innate immunity which play more important role in resistance of larvae to virus. This assumption is based on the assertion that immune function is costly for insects organism that was shown for other species of insects. Since in our experiment insects has been reared on the same diet we suppose that increase of some immune function lead to decrease of other unstudied parameters of innate immunity of insects, more important for viral pathogenesis.

Poster / Viruses. V-13

Apoptosis is induced in haemolymph and fat body of Spodoptera exigua larvae upon oral inoculation with Spodoptera litura nucleopolyhedrovirus

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Spodoptera exigua multiple nucleopolyhedrovirus (SeMNPV) and Spodoptera litura nucleopolyhedrovirus (SpltNPV) are genetically similar but larvae of S. exigua are non-susceptible to SpltNPV. Our goal was to identify if any process was inhibiting SpltNPV infection at some point. S. exigua larvae orally infected with a high concentration of SpltNPV produced a fatal infection in second- or third-instar S. exigua but all dead larvae didn't melt; however, the fourth-instar infected larvae remained healthy. RT-PCR analysis of total RNA from infected second-instar larvae, targeting at immediate early, early, late and very late genes, suggested that the SpltNPV initiated an infection in the non-susceptible hosts. Total DNA extracted from haemocytes of infected larvae showed DNA ladders. Sections of tissue from third-instar infected larvae of S. exigua at 96 h postinoculation stained with haematoxylin and eosin revealed a highly disrupted morphology in fat body. Apoptosis in the fat body tissue was detected employing Terminal Deoxynucleotidyltransferase Mediated Fluorescein-dUTP nick end labeling assays. In situ hybridization assay revealed the presence of viral DNA within the TUNEL-positive area, indicating viral infection in this tissue. We propose that apoptosis limits the viral propagation by reducing the number of SpltNPV-infected haemocytes and fat body cells and inhibits disseminated viral infections.

Poster / Viruses. V-14

Impact of enhancin genes on potency of LdMNPV in oak-fed gypsy moths

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The Lymantria dispar M nucleopolyhedrovirus (LdMNPV) contains two enhancin genes that encode metalloproteases, which may enhance viral potency by degrading key peritrophic matrix (PM) proteins, thereby promoting entry of virus into the midgut epithelial cells to establish primary infections. Previous studies using recombinant viral constructs demonstrated that knocking out both of the enhancin genes resulted in a non- additive decrease in viral potency compared to the potencies of recombinants lacking a single enhancin gene. The bioassay results, in conjunction with early studies by Y. Tanada which indicated a possible cell fusion role for enhancins, suggest that enhancins function both at and beyond the peritrophic membrane (PM). To determine whether one or both of the enhancin proteins have an effect beyond the PM, we first eliminated the PM by supplementing larval food (oak foliage or artificial diet) with an optical brightener, then measured the potency of each of the enhancin deletion constructs administered per os compared with larvae challenged with these constructs in the absence of tinopal. Also, because potency of LdMNPV is markedly attenuated when larvae are fed on oak foliage compared with artificial diet, we investigated whether reduced potency of the enhancin deletion constructs is differentially affected by oak foliar components.

Poster / Viruses. V-15

Functional analysis and baculovirus expression of serpins from the molting fluid of the spruce budworm, Choristoneura fumiferana.

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The vital role that molting has in the insect life-cycle makes it an attractive target for the design of improved and specific pest control agents. To this end, we cloned and characterized in vitro and in vivo four serine protease inhibitors (serpins) (CfSP1, 2, 3 and 4) of C. fumiferana larvae. The four 42 kDa serpin isoforms differed only at their C-terminus, bearing the reactive site loop, and exhibited high protein sequence similarity with the insect Serpin-l family. CfSP1-4 transcripts, expressed mainly in the fat body, were detected in all immature stages. Purified serpins expressed in recombinant baculovirus revealed distinct and specific inhibitory activities toward different proteases. Immunolocalization of the CfSPs proteins demonstrated the presence of the CfSPs in molting fluid and old cuticle, but absent in new cuticle. Three hours after molting, CfSPs were in the cuticle except for CfSP4 which was detectable only in the molting fluid. Functional analysis in 5th instar Trichoplusia ni larvae infected by three recombinant baculoviruses expressing CfSP1, 3 and 4 (AcMNPV-CfSP1, 3 and 4) showed that AcMNPV-CfSP1, 3 and 4 could inhibit the melanization reaction and the cuticle degradation (liquefaction) of 5th instar T. ni larvae during the final stage of infection.

Poster / Viruses. V-16

Effects of Choristoneura fumiferana defective nucleopolyhedrovirus spindlin on viral infectivity and the host's immune genes

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The spindlin protein (GP50) of the Choristoneura fumiferana defective nucleopolyhedrovirus (CfdefNPV) is encoded by a gene present in most baculoviruses but is rarely expressed as heavily as by CfdefNPV. The effects of spindlin on C. fumiferana host gene expression were studied by utilizing cDNA mircoarrays. A virus expressing spindlin and a null mutant were constructed using the Bac-to-Bac baculovirus system. Expression levels of spindlin in Cf203 cells were compared to those of the null mutant at 4 hr, 12 hr and 28 hr post infection. We observed that 29 host genes were significantly up- or down-regulated, being at twofold or greater differential ($P < 0.05$). Two immunorelevant genes including a 6tox and an annexin were significantly down regulated at the late stage of infection. These findings may be related the fact that CfdefNPV spindles enhanced the infectivity of the Amsacta moorei entomopoxvirus in gypsy moth larvae, Lymantria dispar. The above evidence suggests that CfdefNPV spindlin may interfere with the host's immune system. Microarray analysis also showed that the expression level of a chitin synthase gene was suppressed at late stage of infection. Interestingly, In vitro chitin binding assays demonstrated that solublized spindlin was able to bind chitin.

Poster / Viruses. V-17

Importance of peroral infection factors (pif and pif-2) in the interactions between genotypes of Spodoptera frugiperda multinucleopolyhedrovirus (SfMNPV).

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The Spodoptera frugiperda multiple nucleopolyhedrovirus (SfMNPV) genome contains a 17.8 kb that is rich in auxiliary genes and which also codes for two per os infection factor genes: pif and pif-2. Eight plaque-purified genotypic variants from a wild-type (wt) SfMNPV strain isolated in Nicaragua (SfNIC) contain genomic deletions within this region, while only one genotype (SfNIC-B) had the complete genome. Two of the genotypes (SfNIC-C and SfNIC-D), both with similar 16.4 kb deletions, restore the wt pathogenicity when co-occluded with SfNIC-B, which is 2.5 fold less pathogenic than the wt strain. To determine the role of the deleted region and the pif genes in the biological activity of SfNIC, two recombinant viruses were generated using bacmid and plasmid systems and the SfNIC-B genotype as a template. The first one lacked the same 16.3 kb genomic region occurring in SfNIC-C, and was named SfNIC-B16K-null. The second recombinant encompassed a 2.8 Kb deletion including only both pif genes and was named SfNIC-Bpifs-null. Mixed infections of each of these recombinants with SfNIC-B were both 2.4 fold more pathogenic than SfNIC-B alone when the ratio of SfNIC-B to SfNIC-B16K-null or SfNIC-Bpifs-null was approximately 3:1. These results demonstrate that the positive interaction between SfNIC-B and SfNIC-C/D was due to the dilution of the pif genes in the co-occluded infectious mixture. Therefore, an optimized SfMNPV-based bioinsecticide product should contain a genotypic combination such that pif-containing genotypes do not account for higher that 75%.The importance of co-oclussion in baculovirus evolution is discussed.

Poster / Viruses. V-18

Detoxification enzymes of Spodoptera littoralis Boisd. infected with a baculovirus

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Two detoxification enzyme groups (carboxylesterases and Glutathion-S-transferase) were studied in the Egyptian cottonworm (S.littoralis Boisd.) infected with SpliNPV-TR1. Neonates were infected with two different concentrations of SpliNPV-TR1. After infection, larvae were collected once every six hour until first larvae death occured. The carboxylesterases were examined with two substrates (α-naphytl acetate and α-naphytl butyrate) and Glutathion-S-transferases were examined with two substrates (CDNB and DCNB) using a spectrophotometer.The results suggest that detoxification enzyme concentrations were significantly higher in SpliNPV-TR1 infeceted individuls than in uninfected control larvae.

Poster / Viruses. V-19

Characterization of a baculovirus isolated from a diseased larva of Adoxophyes honmai in Japan

Rie Ukuda, Miku Suzuki, Kazuko Nakanishi, Madoka Nakai, Yasuhisa Kunimi

The Baculoviridae comprises two genera, Nucleopolyhedrovirus (NPV) and Granulovirus (GV). The NPVs produce large OBs containing many virions, while the GVs have smaller OBs which contain a single virion. Baculoviruses are considered to be promising candidates for controlling insect pests because they have few negative effects on nontarget organisms and the environment. The smaller tea tortrix, Adoxophyes honmai, is one of the most important pests of tea plants in Japan. A baculovirus was isolated from a diseased larva of A. honmai collected from a tea field in Miyazaki, Japan. OBs of this virus (Mi strain) were cubic in shape, varying in diameter from 0.5 to 2 μm. Most A. honmai larvae infected with Mi strain virus died 10-15 days after molting to the final instar, regardless of the timing of inoculation. Electron microscopic observations showed that OBs of Mi strain contained a single virion. Protein sequence analysis demonstrated that the major protein of Mi strain OBs is most similar to the granulin of A. orana GV. These properties suggest that Mi strain is a mutant of A. orana GV which produces unusually large OBs.

Poster / Viruses. V-20

Pathogenicity and viral multiplication of Xestia c-nigrum granulovirus in larvae of Mythimna separata (Lepidoptera: Noctuidae)

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Baculoviruses, including nucleopolyhedrovirus (NPV) and granulovirus (GV), are specific to invertebrate hosts, mainly lepidopteran larvae. We examined the pathogenicity of Xestia c-nigrum granulovirus (XecnGV) against fifth instar larvae of Mythimna separata. Following inoculation with 100% lethal dose of XecnGV occlusion bodies, the larvae died 12 or more days post inoculation (p.i.), with the median lethal time of 17 days p.i., which is twice more than

the lethal time of general noctuid NPVs. We hypothesize that viral growth in the host cells relates to the viral pathogenesis. Baculoviruses have evolved a biphasic life cycle during which they produce the occlusion derived virus and the budded virus (BV). The BVs released from the primary-infected midgut cells initiate secondary infections in other cells of the host tissues. In order to understand the multiplication of BVs, we measured viral DNA of BVs in the host hemolymph infected with this slow-killing GV using a real-time quantitative PCR. The viral concentration increased quickly and reached a plateau 3 days p.i. Afterward, the infected larvae molted and grew for more than 10 days, but did not pupate. This suggests that XecnGV regulates growth of the host to maximize production of the virus.

Poster / Viruses. V-21

Vertical transmission of ChfuGV into spruce budworm Choristoneura fumiferana sub-lethally infected larvae.

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Several studies show the great variations in the efficacy of baculovirus to control populations of C. fumiferana. Following observation of ChfuGV viral particles inside gonads of C. fumiferana larvae infected seven days before, a study of vertical transmission was realized in fourth instars larvae. Using a sub-lethal concentration, an histopathological study, where Azan stain was applied, shown the presence of the granulovirus into survival-infected larvae, into ovaries of females adults, and into hatching filial larvae (F1). Also, a PCR detection of P10 protein associated to ChfuGV was also performed in order to confirm the presence of the ChfuGV into infected larvae, females, eggs and filial larvae.

Poster / Viruses. V-22

Relative frequency of HaSNPV and HzSNPV nucleopolyhedrovirus after multiplication in different hosts.

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The family Baculoviridae is a large group of arthropod-specific viruses that infect mainly species within the order Lepidoptera. This is the case of the HaSNPV and HzSNPV, two highly related nucleopolyhedrovirus that infect heliothines (Noctuidae). These two viruses are very similar in their nucleotide (97% identity) and amino acid (99% identity) sequences and some authors consider them as two isolates of the same virus. In our laboratory, in an epizootic outbreak in an insect population of H. zea, larvae from this specie were infected with a laboratory preparation of HaSNPV ***(HaSNPV_P1).*** Viral particles from infected insects were isolated (HaSNPV_P2) and analyzed. Sequence of lef-8 and polh genes were obtained identifying the presence of the HzSNPV virus in high frequency in HaSNPV_P2 isolated. Additional test showed the presence of the HzSNPV, although in a lower frequency, in the inoculum that started the epizootic infection (HaSNPV_P1). In the present work, we show the identification and characterization of these isolates according to the biological activity as well as the effect of the host in their relative frequency.

Poster / Viruses. V-23

Modulation of host gene expression in a spruce budworm cell line infected by wild type or recombinant Choristoneura fumiferana nucleopolyhedrovirus.

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The impact of genetically-modified biocontrol agents on expression of pest insect genomes is at present poorly understood. We have studied gene expression changes in Cf203, a Choristoneura fumiferana cell line infected with either a wild type or a modified strain of the C. fumiferana multiple nucleopolyhedrovirus (CfMNPV) using DNA microarray technology. The modified strain (recCfMNPV) has been engineered by inserting the Choristoneura hormone receptor 3 (CHR3) cDNA in the locus encoding the ecdysteroid UDP-glucosyltrasferase (egt). The temporal regulation of 5000 C. fumiferana unigenes in the Cf203 cell line was tracked following infection with either wild type or recCfMNPV. In wild type CfMNPV infected cells, host gene expression was significantly different at early, late and very late times post infection in comparison with time = 0. The differentially up- and down- regulated host genes in the early stages of infection were involved in cell signaling, cell proliferation and transcriptional regulation. However, for the majority of host genes, mRNA levels were drastically reduced at very late times post infection. Compared with the wild type CfMNPV, infection with recCfMNPV altered the expression of different host genes including transcription factors, translation initiation factors, heat shock proteins as well as uncharacterized genes.

Poster / Viruses. V-24

Expression of foreign protein by a polyhedrin-positive, cathepsin null recombinant baculovirus.

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There is great potential to use baculovirus expression vector systems (BEVS) for producing foreign proteins in lepidopteran larvae. Most commercial BEVS are engineered to insert foreign genes into the polyhedrin (polh) locus and lack the polh gene. These viruses cannot produce occlusion bodies and are inconvenient for per os inoculation of larvae. Current knowledge in baculovirus genomics makes it possible to engineer BEVS into other parts of the virus genome.

The baculovirus cysteine proteinase, V-CATH is involved in host liquefaction post mortem and is not required for virus replication. Host liquefaction is undesirable for in vivo protein production and V-CATH proteolytic activity can degrade foreign proteins. Thereupon we have engineered a BEVS to insert foreign genes into the v-cath locus of the Bombyx mori Nucleopolyhedrovirus (BmNPV) such that the v-cath gene is deleted and the native polh gene is retained. This BEVS also uses “Homingbac” direct cloning technology we recently developed which permits insertion of foreign genes without intermediate plasmid or bacteriophage steps.

In our work, we have expressed recombinant M-HBsAg (middle surface antigen of human hepatitis B) in the baculovirus construct, rBmNPV-Δv-cath-M-HBsAg. Silkworm larvae were infected per os and M-HBsAg was observed to be abundantly produced at a very late stage of infection. Foreign protein remained present in high quantity and abundance after death of larvae. The v-cath locus is an excellent alternative to the polh locus when a BEVS is intended for foreign protein production in lepidopteran larvae.

Poster / Viruses. V-25

Construction of advanced baculovirus expression vector for generating high-throughput recombinant proteins

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A number of methods for the convenient and rapid generation of recombinant baculoviruses expressing gene of interest have been reported so far. However, the major drawback of baculovirus expression system is the tedious efforts required to purify recombinant virus from non-recombinant backgrounds. For the easy generation of pure recombinant baculovirus, extracellular RNase gene from Bacillus amyloliquefaciens, barnase, was introduced into Autographa californica nucleopolyhedrovirus (AcNPV) genome under the control of Cotesia plutellae bracovirus (CpBV) ORF3005 promoter. In addition, bacteriophage lambda site-specific attachment (att) sites were also integrated to AcNPV genome to improve the efficiency and specificity of recombination. Insect cells transfected with this modified baculovirus expression vector, named EasyBac, showed slightly cytopathic effects with reduced cell growth after transfection. Both co-transfection and in vitro transposition of the EasyBac with pAc-LacZ, which has not only homologous recombination regions but also att sites, yielded β-galactosidase through the substitution of the barnase gene with lacZ gene by both in vivo homologous recombination and in vitro site specific transposition. Moreover, no non-recombinant backgrounds were detected from unpurified recombinant stocks. These results suggest that the EasyBac has an effective benefit enabling for high-throughput baculovirus expression vector without purifying recombinant virus.

TUESDAY, AUGUST 14TH

CONTRIBUTED PAPERS, Tuesday 8:00 - 10:00

MICROBIAL CONTROL I

Symposium. Tuesday, 8:00. (29)

Efficacy of mixtures of Beauveria bassiana strains in the control of coffee berry borer, under laboratory and field conditions

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Under laboratory and Colombian coffee field conditions the mortality caused by seven Beauveria bassiana strains and a mixture of high virulence strains and low virulence strains against the Coffee berry borer (CBB), Hypothenemus hampei (Ferrari) was determined. Under laboratory conditions the mortality caused by the treatments was measured by immersion of CBB in 1 x 106 spores/ml suspension. In the field, each treatment was sprayed in plots of 25 trees with 10 repetitions. In each plot, one coffee tree and one branch with 50 coffee berries were artificially infested with 150 adult CBB. After 24 h, the infested branches were sprayed with 1 x 109 spores/tree/treatment. Mortality of insects was evaluated 30 days after spraying. Under lab conditions the highest mortality (100%) was obtained with the mixture of low virulence strains, same results were obtained under field conditions, reaching a mortality of 66.6%. The lowest percentage of mortality in lab was 53.3% with the strain Bb9024. However in field, the lowest mortality was 53.1%, with strain Bb9020. In conclusion the mixture of low virulence strains, always produced highest mortality. Results indicate the promising potential of designing strain mixtures as an alternative to biocontrol of CBB and other pests.

Symposium. Tuesday, 8:15. (30)

Differential gene expression by Metarhizium anisopliae grown on plant root exudate

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Metarhizium anisopliae has been isolated from two sources: infected insects and soil. M. anisopliae survives best in rhizospheric soils where 105 propagules/g have been found 6 months after initial soil applications. Our experiments evaluated the germination of spores from 11 entomopathogenic fungi (Beauveria and Metarhizium spp.), and 2 soil saprophytic fungi (the non-rhizospheric fungus Aspergillus niger and the rhizosphere competent Trichoderma harzianum) at several concentrations of bean root exudates (RE) (1, 2.5, 5, 10 and 20 mg/ml). Germination rates ranged from 5 to 98% with 1 mg/ml of RE. At lower concentrations of RE M. anisopliae (ARSEF 2575) showed higher germinations rates than T. harzianum. Using microarrays, we identified the subset of genes that M. anisopliae expressed after 1, 4, 8 and 12 hours of incubation at 5 mg/ml of RE. Up- and down- regulated genes were organized into functionally related groups. Results showed that most up-regulated genes were involved in cell metabolism, cell structure, stress response/defense and protein/energy metabolism. A large number of genes with unknown function were also either up-or down-regulated on RE indicating that many previously uncharacterized genes may have functions related to saprophytic survival, which need to be determined.

Symposium. Tuesday, 8:30. (31)

Biological control of Hoplia philanthus (Coleptera: Scarabaeidae) using entomopathogenic nematodes

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Larvae of the scarabaeid beetle, Hoplia philanthus cause severe damage in lawns, turf, pastures and ornamental crops in Belgium. The biological control potential of nine different entomopathogenic nematodes (EPN); Heterorhabditis bacteriophora, H. megidis, H. indica, Steinernema scarabaei, S. feltiae, S. arenarium, S. carpocapsae Belgian strain, S. glaseri Belgian and NC strain was tested against 2nd, 3rd instars larvae and pupae of H. philanthus in laboratory and greenhouse. The S. glaseri Belgian strain, S. glaseri NC strain, H. bacteriophora and H. megidis were highly pathogenic to both 3rd, 2nd instar larvae and pupae. S. glaseri Belgian strain and NC strain caused greater mortality of 2nd instar (60-80% mortality and LD50 768.82-950 IJs/grub) and 3rd instar (90-100% mortality and LD50 89.96-133.74 IJs/grub) larvae comparable to the mortality of pupae (93-100%) caused by H. bacteriophora, H. megidis, S. glaseri Belgian strain, and NC strain. In Pot experiments (2.5 and 5.0 billion ha-1 doses) H. bacteriophora, H. megidis, S. glaseri and S. scarabaei were highly pathogenic to 2nd (75-97% mortality) and

3rd instar (80-82.5%) larvae. This study proved variation in pathogenicity of EPNs to white grub and H. bacteriophora, H. megidis, S. scarabaei and S. glaseri seem to be promising candidates for biological control.

Symposium. Tuesday, 8:45. (32)

Synergistic interaction in white grubs control

Anuar Morales, Daniel Peck.
Cornell University

Root-feeding white grubs are the most important insect pests in turfgrass, but best IPM is hampered by reliance on early-season preventive insecticides. Recent research, however, reveals that synergies between specific combinations of biological and chemical controls might open opportunities for curative control. To further explore the extent and potential of synergisms, we tested the interaction between nine entomopathogenic fungi, nematodes and bacteria with sublethal doses of two neonicotinoid insecticides (imidacloprid and clothianidin) against third instar European chafer and Japanese beetle under laboratory and greenhouse conditions. In laboratory bioassays, the type of interaction varied with grub species, insecticide and biological. In European chafers, the interaction between both insecticides and Heterorhabditis bacteriophora was synergistic, but for imidacloprid and Metarhizium anisopliae it was additive. In Japanese beetle, the interaction between Beauveria bassiana and clothianidin was synergistic, additive for both insecticides and Paenibacillus popilliae, and antagonistic with both insecticides and Bt tenebrionis. Similar results were obtained in greenhouse pot trials but overall rates of control were lower. Future studies will advance the most promising combinations to field trials wherein the ultimate goal is a late-season curative that permits the assessment of thresholds, and features a biological in tandem with a low-rate of a reduced-risk chemical insecticide.

Symposium. Tuesday, 9:00. (33)

Pink Bollworm Resistance to Bt Cotton: Still Rare After All These Years

Bruce E. Tabashnik, Jeffrey A. Fabrick, Shai Morin, Mark S. Sisterson, Yves Carrière, and Timothy J. Dennehy

Transgenic crops producing Bacillus thuringiensis (Bt) toxins reduce reliance on insecticides, but evolution of resistance by pests could cut short their usefulness. Pink bollworm is a major pest that has experienced intense selection for resistance to Bt cotton in Arizona since 1997. Unexpectedly, bioassay data show that the frequency of pink bollworm resistance to Cry1Ac, the toxin in Bt cotton, decreased from 1997 to 2006. Field-based estimates also show sustained efficacy during this period. In laboratory-selected strains that survive on Bt cotton and have up to 3,100-fold resistance to Cry1Ac, resistance is linked with three recessive mutations in the gene encoding a cadherin protein that binds Cry1Ac. Each of the three resistant alleles has a deletion upstream of the toxin-binding region of the cadherin protein. We developed a PCR-based method for detecting each of the three alleles to monitor resistance. Screening of DNA from >6,600 insects from 79 cotton fields during 2001 to 2006 detected no resistance alleles. These results show pink bollworm resistance remained rare despite a decade of exposure to Bt cotton, contradicting predictions of rapid pest resistance to Bt crops. These results have important implications for efforts to eradicate pink bollworm from Arizona and neighboring areas.

Symposium. Tuesday, 9:15. (34)

Fungal biopesticides for sucking pest management in Australian broadacre crops

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1 Department of Primary Industries & Fisheries, Queensland, Australia
2 Current affiliation: Monsanto Australia.

Sucking pests are an emerging threat to conventional and GM crops in Australia. The use of broad-spectrum chemical insecticides to control early season sucking pests such as Creontiades spp. (Hemiptera: Miridae) threatens successful insecticide resistance management strategies and disrupts natural enemy populations, with potential to trigger outbreaks of other pests such as multiple-insecticide resistant silver-leaf whitefly, Bemisia tabaci.

The successful adoption of baculovirus biopesticides against lepidopteran pests in Australian has resulted in industry demand for biopesticides for sucking pest management. Isolates of Metarhizium anisopliae have been identified with activity against a range of sucking pests in cotton, grain and pulse crops, and field trials have demonstrated consistent efficacy.

Speed of kill under field conditions using Metarhizium is relatively rapid, with pest populations beginning to decline by 3 days and significant control by 6 days after application. Impacts on natural enemies are negligible. Formulation in oil and application by ultra-low volume spray has proved more effective that formulation in emulsified oil or conventional spray equipment.

Further research on timing, rates and formulation of Metarhizium against sucking pests in pulse and grain crops continues using an action learning model with growers and consultants to directly address industry requirements during product development.

Symposium. Tuesday, 9:30. (35)

Use of Beauveria bassiana and imidacloprid for control of emerald ash borer in an ash nursery

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We wish to determine the potential of Beauveria bassiana (strain GHA), alone or in combination with imidacloprid, for control and management of emerald ash borer (EAB), Agrilus planipennis. Using a commercial tree nursery in southern Michigan we treated approximately 400 Fraxinus pennsylvanica and F. americana (height ca. 5-6 m) with fungus alone, imidacloprid alone at two rates, fungus plus the low rate of imidacloprid, or a formulation blank as control. Imidacloprid (Bayer) was applied as an early season drench in late May, and the fungus (BotaniGard ES, Laverlam) and formulation blank were applied three times in June and July. We monitored spore deposition and estimated spore persistence on leaves and bark. We will continue monitoring fungal survival throughout the study. Initial EAB infestation was low, but we observed beetle activity within our plots and on ash trees nearby. At least 4 genotypes of B. bassiana were present in soil before any sprays and none of them was strain GHA. After sprays, we readily reisolated strain GHA from leaves (up to 6 weeks post-spray), bark and soil. Adults trapped within the plots were infected with the GHA strain. We have completed 2 seasons of a multi-year study.

Symposium. Tuesday, 9:45. (36)

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

Jorgen Eilenberg1, Gisbert Zimmermann2, Kerstin Jung2, Tariq Butt3, Charlotte Nielsen1, Milton Typas4.
1.University of Copenhagen, Faculty of Life Sciences, 2.BBA, Institute for Biological Control, 3.Department of Biological Sciences, University of Swansea, 4.University of Athens, Department of Genetics and Biotechnology,

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

SYMPOSIUM, Tuesday 8:00 - 10:00

VIRUS DIVISION Symposium II: to Honor Loy Volkman. Baculovirus Bounty

Symposium. Tuesday, 8:05. (37)

Viruses Insex and the SIP

John P. Burand.
Department of Plant Soil & Insect Science,
University of Massachusetts - Amherst, MA.

Understanding the interactions that occur between viruses and their insect host is key to unravelling pathology resulting from infection with insect pathogenic viruses. Over the past 20 years, research in my laboratory has focused on studying virus:host interactions including the regulation of ecdysone titer in baculovirus infected insects and the principle steps in entry of baculoviruses into insect midgut cells leading to the establishment of primary infections of the host. By far the most intriguing virus:host interactions we have studied to date are those resulting from the infection by the sexually transmitted insect virus, Hz-2V. Replication of this virus in the reproductive tissues of infected females results in the proliferation of infected cells, hypertrophy of these tissues and increased pheromone production by infected female moths. Here I present information of the differential expression of viral genes in these infected tissues and discuss the potential use of novel mechanism by Hz-2V which may regulate both host and viral gene expression.

Symposium. Tuesday, 8:30. (38)

Functional analysis of the interaction between BmNPV ORF8 and its host factors

WonKyung Kang
Molecular Entomology Laboratory, RIKEN, Wako, JAPAN

The Baculoviridae is a large family of viruses that infect invertebrates, particularly lepidopteran insects. Baculoviruses are divided into two genera on the basis of occlusion body morphology, the nucleopolyhedroviruses (NPVs) and the granuloviruses. Based on phylogenetic studies the lepidopteran NPVs can be further subdivided into groups I and II. The orf8 gene of Bombyx mori NPV (BmNPV) is one of a set of genes unique to group I NPVs. Our laboratory has demonstrated that the BmNPV orf8 gene encodes a nuclear protein that plays an important role during viral infection. We also showed that ORF8 colocalized with IE1 to specific nuclear sites throughout the infection and that IE1 and hr facilitate this localization, suggesting that ORF8 is one of the viral components that occupy nuclear specific regions for viral DNA replication. To investigate further, host proteins interacting with ORF8 were screened using a yeast two-hybrid system. Three cDNA libraries derived from BmN cells or insect larvae were used and several independent clones were obtained. The characterization of these host factors and further analysis of the interaction between ORF8 and host factors during the BmNPV infection cycle will be discussed.

Symposium. Tuesday, 8:55. (39)

Expanding baculovirus bounty through glycoengineering.

Donald L. Jarvis, Department of Molecular Biology, University of Wyoming, Laramie, WY 82071

The baculovirus-insect system is widely used for recombinant protein production. This system can provide high-level production and eukaryotic protein modifications, such as glycosylation. However, insect protein glycosylation pathways are not identical to those of higher eukaryotes. Therefore, the baculovirus-insect cell system typically fails to produce recombinant glycoproteins with oligosaccharide side chains identical to those found on native, higher eukaryotic products. For the past decade, we have been addressing this problem by using genetic engineering of both the baculovirus vector and the insect cell host in an effort to humanize the protein N glycosylation pathway in this system. This presentation will focus on these efforts with an emphasis on transgenic insect cell lines with humanized N-glycosylation pathways.

Symposium. Tuesday, 9:20. (40)

Baculovirus replication sites: role of cellular and viral genes

Dagmar Knebel-Mörsdorf
Department of Neurology and Center for Biochemistry, University of Cologne, Cologne, Germany

The genome of the baculovirus Autographa californica multicapsid nucleopolyhedrosis virus (AcMNPV) is replicated in the host cell nuclei and viral DNA synthesis takes place in discrete regions. Rearrangement of nuclear structures can start early during infection and become extreme as viral proteins and replicating DNA accumulate in replication compartments to fill the nucleus. Several viral factors are recruited to viral replication compartments such as IE2, LEF-3, and DBP. The impact of these factors has been addressed by RNA interference indicating that replication sites are still formed when IE2 and DBP are silenced. Less is known about cellular factors that are recruited to these nuclear structures. We observed at least one factor, the TATA binding protein that colocalizes with replication compartments. These results suggest that both viral DNA synthesis and late viral transcription take place at common nuclear structures.

(Supported by the Deutsche Forschungsgemeinschaft and the Köln Fortune Program/Faculty of Medicine, University of Cologne)

Symposium. Tuesday, 9:55. (40,1)

The Race to Death: The interplay between the insect immune defenses and the entomopathogenic nematode cuticle in determining host specificity

Diana L. Cox-Foster, Xinyi, Li, and Erin Troy. Department of Entomology, Penn State University, University Park, PA, USA

Infective juveniles (IJs) of entomopathogenic nematodes (EPNs) penetrate insect hosts and release symbiotic bacteria that kill the hosts. Insect hosts defend against EPNs by rapid cellular immune responses that result in encapsulation and melanization, which can kill EPNs. Nematodes have to overcome the innate immunity of the hosts to survive and reproduce and the release of symbiotic bacteria (EPB) has to occur before the intensive host immune responses occur. The cellular immune response was investigated in multiple hosts against several species. Surface coat proteins (SCPs) of EPNs play a role in the suppression/evasion of host immune responses. We demonstrated that different species and strains of EPNs have different surface coat protein profiles. SCPs from Steinernema glaseri NC strain were isolated and characterized; these SCPs suppressed immune responses of hosts, in a species-specific manner corre-

sponding to the infectivity of the hosts by the intact nematode/bacteria. The mechanisms employed by entomopathogenic nematodes and discovered to date will be discussed and compared to those utilized by other parasitic nematodes.

IFENSB Session II, Tuesday 8:00 - 10:00:

VIRULENCE

Symposium. Tuesday, 8:00. (41)

The Race to Death: The interplay between the insect immune defenses and the entomopathogenic nematode cuticle in determining host specificity.

Diana L. Cox-Foster, Xinyi, Li, and Erin Troy. Department of Entomology, Penn State University, University Park, PA, USA

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Symposium. Tuesday, 8:30. (42)

Neuroimmunity: insights from the C. elegans pathogenesis model

Man-Wah Tan, Trupti Kawli and Eric Evans
Department of Genetics, and Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA 94305, U.S.A.

The innate immune system provides a critical first line of defense against pathogens. It includes molecules and signaling cascades local to the infected tissues, and those produced by neighboring tissues. Recent studies have provided compelling evidence that the innate immune system is evolutionarily conserved; it involves the recognition of pathogen-associated molecular patterns, or infection by-products by receptors leading to regulated expression of immune modulators and antimicrobial molecules. A wealth of data has also indicated that the nervous system regulates innate immunity through hormonal and neuronal routes. However, the precise mechanisms and physiological circumstances under which the neuroendocrine system modulates the innate immunity in the context of a whole organism remain poorly defined.

The C. elegans pathogenesis model is uniquely suited to investigate how the neuroendocrine system modulates immune function in vivo. In addition to the powerful genetic and genomic tools, because most mutants defective in neuronal function are viable, we are able to visualize and quantify infection within a live host. We find that the C. elegans nervous system modulates immune function in part through the insulin pathway. P. aeruginosa in turn acts on this pathway to suppress immune function. We will discuss the molecular mechanisms underlying this interaction.

Symposium. Tuesday, 9:00. (43)

Exploiting the Photorhabdus genome

R. ffrench-Constant1, A. Dowling1, M. Hares1, J. Parkhill2 and N. Waterfield3
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We now have two finished genomes of Photorhabdus, P. luminescens and P. asymbiotica. We will describe the insecticidal toxins present in each genome and discuss what we know about their biology and mode of action. We will also describe novel approaches for the rapid screening of bacterial genomes for proteins or compounds active against invertebrates (insects, nematodes or amoebae). Such approaches look

set to increase the already growing number of insecticidal toxins produced by these two species, including the Toxin complexes (Tc's), Makes caterpillars floppy toxins 1 and 2 (Mcf1 and Mcf2) and the PirAB toxins.

Symposium. Tuesday, 9:30. (44)

Virulence of entomopathogenic nematodes and symbiotic bacteria complexes

Parwinder Grewal
Department of Entomology
Ohio State University, Wooster, OH 44691

Virulence, the disease producing ability, of entomopathogenic nematodes and symbiotic bacteria complexes depends upon a multitude of factors. Ecological, behavioral, physiological, and molecular interactions among the nematodes, bacteria and the hosts influence the virulence the nematode-bacteria complex. The infective juvenile nematodes carrying the symbiotic bacteria in their guts persist in the soil in search of a susceptible insect host. The infective juveniles find suitable hosts using an array of host-related cues. The infective juveniles then attempt to attach to the host to penetrate through the host cuticle or natural body openings either mechanically or by producing proteases. At this stage the host under attack launches a suite of evasive and defensive behaviors that may limit infection by the nematode-bacteria complex. Studies on white grubs indicate that grub species use diverse and effective evasive and defensive behaviors include scraping, brushing, rubbing, and chewing that can prevent nematode attachment and penetration and often killing the invading nematodes.

SYMPOSIUM, Tuesday 8:00 - 10:00:

Cross-Divisional: Current situation on the biological control of turfgrass insects

Symposium. Tuesday, 8:00. (45)

Entomopathogenic nematodes for pest management in turfgrass: discovery, development, and implementation

Parwinder Grewal
Department of Entomology
Ohio State University, Wooster, OH 44691

Application of chemical insecticides has been the main method of defense against damage by turfgrass pests. However, many of the chemical insecticides used for turfgrass pest control have been taken out of the market while others are under scrutiny by the United States Environmental Protection Agency due to the proposed implementation of Food Quality Protection Act (FQPA). Local ordinances and public opinion has further restrained the use of the remaining products in Canada and some parts of USA. Therefore, turfgrass managers have few options for curative control of turfgrass pests. Entomopathogenic nematodes are effective biological control agents of most turfgrass pests. The lack of consistency in pest control has been the major hurdle

in the adoption of nematodes by golf course superintendents and lawn care companies. Tremendous progress has been made in the past ten years in the identification of more virulent nematode species and strains particularly against white grubs. These new nematode strains, H. bacteriophora GPS11 and TF, H. zealandica X1, and S scarabaei AMK001, have shown increased consistency in white grub control. These strains provide equal or better curative grub control than the most commonly used chemical insecticides. Two strains, H. bacteriophora GPS11 and H. zealandica X1 have already become commercially available in the USA and Australia, respectively. Unfortunately, S. scarabaei has proven difficult to mass-produce with established nematode mass-production technology (Ralf Ehlers, personal communication, Germany). Nematodes are currently used for the control of white grubs, crane fly and dog fleas, billbugs, and mole crickets in turfgrass in the USA and Canada. Small lawn care companies particularly those that provide organic or natural lawn care have begun to use nematodes to manage white grubs and billbugs. In Australia, the nematodes are used for white grub control on public properties such as urban parks. In Japan, the nematodes are applied for the control of billbugs and white grubs on golf courses and in Europe, the nematodes are used mainly for the white grub control on golf courses. Results on field efficacy testing and application technology development will be presented.

Symposium. Tuesday, 8:20. (46)

Fungi for the Control of Turfgrass Insects: An Overview

Rick Brandenburg, Dept. of Entomology
N. C. State University, Raleigh, NC USA

Insects as pests of turgrass have been a consistent and more recently, an increasing problem, in our efforts to maintain high quality turfgrass. High quality turfgrass is often located in environmentally-sensitive areas. Recent research and attempts to market fungal pathogens have focused on Beauveria bassiana and Metirhizium aniplosae. General acceptance in the field has been limited due to performance that was inconsistent. Factors that appear to limit the efficacy of these fungal pathogens include spore viability over time (due to heat, UV degradation etc), strain specificity for specific hosts, and issues associated with producing high quality material that lacks contamination. Other factors that influence the efficacy and performance of these pathogenic fungi include the behavior of the insect pest relative to the presence of the fungi. Research has demonstrated that certain soil insect such as white grubs and mole crickets. Strains of the pathogen that demonstrate high efficacy against a certain insect pests also produces a strong repellency effect on that insect. The insect modifies its behavior to avoid areas in the soil where the fungal spores are present.

Symposium. Tuesday, 8:40. (47)

Prospects for managing turfgrass insects with baculoviruses

Callie A. Prater1 and Daniel A. Potter2
1 North Carolina State University, Department of Entomology, Campus Box 7613 Raleigh, NC 27695
2 University of Kentucky, Department of Entomology, S-225 Agric. Science Bldg. N, Lexington, KY University of Kentucky, Lexington, KY 40546

Mounting public pressure for safer pesticides, increased restrictions and loss of insecticide registrations, as well as concerns of insecticide resistance have lead to increased interest in microbial insecticides for suppressing insect pests of lawns, golf courses, and sports fields. Viruses, in particular baculoviruses, have shown promise as bioinsecticides in other systems due to their specificity, virulence, and persistence. A naturally occurring baculovirus, Agrotis ipsilon multicapsid nucleopolyhedrovirus (AgipMNPV), was recently discovered decimating black cutworm (Agrotis ipsilon Hufnagel) populations on several central Kentucky golf courses. Laboratory, greenhouse, and field trials showed that AgipMNPV has good promise as a bioinsecticide for black cutworm management in turfgrass. This study is the first to evaluate the use of a virus against a pest in turfgrass. This talk will address the results of the aforementioned investigations as well the prospects and limitations for baculovirus development for the turfgrass market.

Key Words Black cutworm, Agrotis ipsilon, baculovirus, Agrotis ipsilon multicapsid nucleopolyhedrovirus, turfgrass

Symposium. Tuesday, 9:00. (48)

Combination of entomopathogenic nematodes with other control agents for the management of white grubs in turfgrass

Albrecht M. ¬Koppenhöfer
Dept. Entomology, Rutgers University, Blake Hall, 93 Lipman Dr., New Brunswick, NJ 08901

Most combinations of nematodes with other control agents have been studied against 3rd instar white grubs (Coleoptera: Scarabaeidae). Combination of 2 nematode spp., i.e., Heterorhabditis bacteriophora, Steinernema kushidai, or S. glaseri, against Cyclocephala hirta or Anomala orientalis, generally resulted in additive effects. Milky disease bacterium, Paenibacillus popilliae, infection of C. hirta enhances H. bacteriophora and S. glaseri efficacy by facilitating nematode penetration into the midgut with no negative effects on either agent's reproduction. Combinations of S. glaseri or H. bacteriophora with Bacillus thuringiensis ssp. japonensis results in additive or synergistic effects on mortality of Cyclocephala spp., Popillia japonica, and A. orientalis with reproduction of H. bacteriophora but not of S. glaseri being reduced in C. hirta. The neonicotinoid imidacloprid reduces white grub defensive behaviors resulting in synergistic interaction with Heterorhabditis spp. and S. glaseri without compromising nematode reproduction. Of all these combinations, those with imidacloprid have provided the most consistent synergism and are also economically the most feasible. Recent observations indicate that application of these combinations against younger larvae (2nd and early 3rd instars) allows further reduction of nematode and imidacloprid rates, thus increasing the combinations' feasibility.

Symposium. Tuesday, 9:20. (49)

Biological control of turfgrass insect pests in Canada – current situation.

Louis Simard and Guy Bélair
Horticulture Research and Development Centre, Agriculture and Agri-Food Canada, St-Jean-sur-Richelieu, Quebec, Canada.

Turfgrass management in Canada is currently under pressure to reduce the use of chemical pesticides and to undertake a more aggressive consideration of biological agents. However, there are presently few biological control products available or registered to control turfgrass insect pests. In 2002, 2003 and 2005, the first extensive entomopathogenic nematodes (EPN) and fungi (EPF) soil surveys in turfgrass were conducted in two Canadian provinces, Québec and Ontario. Twenty-five EPN isolates (prevalent species Steinernema carpocapsae and S.feltiae) and more than 200 EPF isolates (prevalent species Metarhizium anisopliae) were found on golf courses. Studies performed on the black cutworm (Agrotis ipsilon) and the European crane fly (Tipula paludosa) showed differences regarding the virulence of our Canadian biological agent isolates against these insect pests. Other potential agents such as Saccharopolyspora spinosa and Beauveria bassiana were tested against the black cutworm and show some promises as biological control management tools. Presently, few researchers are working on biological control of turfgrass insect pests in Canada. Thus, the development and the implementation of new bio-products are urgently needed to maintain our current standards of turfgrass quality requested by this industry.

TUESDAY AM

CONTRIBUTED PAPERS, Tuesday 10:30 - 12:30

BACTERIA 1

Contributed Paper. Tuesday, 10:30. (50)

Death caused by Bacillus thuringiensis is followed by extensive colonization and sporulation in natural and certain atypical hosts

Brian A. Federici
Department of Entomology
and Graduate Programs in Genomics and Molecular Biology
University of California-Riverside, Riverside, CA 92521, USA

The insecticidal bacterium, Bacillus thuringiensis (Bt) consists of numerous subspecies that produce insecticidal crystal proteins during sporulation. These proteins are toxic to insects, especially to larvae of lepidopteran species. The efficacy of certain Bt isolates, such as the HD1 isolate of B. thuringiensis subsp. kurstaki, is so high against many lepidopteran pests that it is the most successful microbial insecticide developed to date. Moreover, two of the Cry proteins, Cry1Ab and Cry1Ac, that account for larval intoxication, are now used widely in transgenic crops, mainly Bt corn and Bt cotton. Despite the commercial success of insecticides and Bt crops, and the existence of numerous insecticidal subspecies of Bt, epizootics caused by this bacterium are rare, and typically have only been reported in larval populations of larvae of grain-feeding moths. This led some workers recently to question the ability of Bt to kill and colonize lepidopteran larvae. Instead, these worker suggested that native midgut bacteria cause larval death in lepidopteran species. To test this hypothesis, sporulated cells of HD1 (25,000 spores/larva) were fed to 2nd and 3rd instars of several lepidopteran species including those of the naval orangeworm, Amyelois transitella (Pyralidae) and Tricoplusia ni (Noctuidae). At this dose, 80-100% of the larvae were killed by a combination of Cry protein intoxication followed by invasion, colonization, and sporulation of Bt. Plating of bacteria on nutrient agar after death showed the larval cadavers to be virtually pure cultures of HD1, with rates of reproduction averaging 3,000-fold. These results show that HD1 alone is fully capable of causing larval death through an infection and colonization process, and is highly effective at using the nutrients in larval cadavers for extensive reproduction and sporulation.

Contributed Paper. Tuesday, 10:45. (51)

The genomic structure of three Lepidoptera cadherin-like Bacillus thuringiensis related genes

Yolanda Bel and Baltasar Escriche
Genetics Department, University of Valencia,Dr Moliner, 50, 46100, Burjassot, Valencia, Spain

In some Lepidoptera, insect resistance to Bacillus thuringiensis Cry toxins is associated with a cadherin-like protein present in the midgut epithelial cells. We have studied the structure of the genes that encode these cadherin-like proteins in Ostrinia nubilalis, Helicoverpa armigera, and Bombyx mori. The results show that the size of the genes is 19.6 kb, 20.0 kb, and 41.8 kb respectively. The variation is due to differences in the intron sizes. In contrast, the sizes of the exons are almost completely preserved among the three species, because the intronic sequences (except the first and last one) are inserted in homologous positions in the respective cDNA sequences. The three genes are composed by 35 exons linked by 34 introns and, in the three species, the first intron (in the 5'-untranslated region) is the longest one. Partial sequences from transposable elements found only in B. mori introns explain, in part, the largest size of its gene. The results point out a highly conserved structure that indicates that these genes are orthologous. The in silico analysis of the protein products of these genes (grouped into the protocadherin family) shows a common structure with 12 cadherin repeats, a transmembrane region and a short intracellular domain.

Contributed Paper. Tuesday, 11:00. (53)

Identification and characterization of compounds involved in the stimulation of cry1Aa expression of Bacillus thuringiensis var. kurstaki

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In a previous work, we identified the peptide SKPDT (NprRB) in the supernatant of the transition phase of Bacillus thuringiensis var. kurstaki. Transcription of cry1Aa, sporulation and spore release were stimulated when chemically synthesized SKPDT was added to the culture during the transition phase. Indeed, cry1Aa was transcribed 3-folds mainly from the late promoter BtII, which requires the late-stage sporulation specific transcription factor σK. Thus, SKPDT is a signaling peptide for B. thuringiensis. SKPDT did not affect the growth. To establish a quick screening method for signaling peptides, SKPDT was used to evaluate the effect in starved cells of B. thuringiensis (BtpHTcry1A2, strain containing a cry1Aa promoter-lacZ fusion). Cells were collected during the exponential phase and put on sterile paper disks, containing SKPDT or the whole supernatant of the transition phase, on agar Petri dishes. Colonies after 22 h became blue and the color was five times deeper in disks containing supernatant than in those containing the peptide. We suppose that besides the SKPDT, other peptides or substances act synergistically. Currently, we are working on the identification and characterization of other excreted compounds involved in the regulation of the sporulation process and cry1Aa expression.

Contributed Paper. Tuesday, 11:15. (54)

The pre-pore oligomer is an obligate intermediate in the cell death induced by Bacillus thuringiensis Cry1Ab toxin in insect larvae.

Nuria Jimenez-Juárez, Isabel Gómez, Ivan Arenas, Liliana Pardo, Carlos Muñoz-Garay, Sarjeet S. Gilla, Alejandra Bravo and Mario Soberón.
Instituto de Biotecnología, Universidad Nacional Autónoma de México. Apdo. postal 510-3, Cuernavaca 62250, Morelos, Mexico, and aDepartment of Cell Biology and Neuroscience, University of California, Riverside, CA 92506.

Cry toxins of Bt are pore forming toxins, their primary action is to lyse midgut epithelial cells in target insect. In Manduca sexta, a cadherin-like protein (Bt-R1) and aminopeptidase-N (APN), were described as Cry1A-receptors. Previously, we showed that binding of monomeric Cry1Ab toxin to Bt-R1 promotes the formation of a pre-pore oligomeric structure that is competent in membrane insertion. The oligomeric Cry1A structure then binds to APN receptor leading to its insertion into membrane lipid rafts implying a sequential binding mechanism of Cry1A toxins with Bt-R1 and APN. In this work we will present two sets of data that support the pore-forming model. We identified antibody (scFvM22) that recognizes β16-β22 in domain III. ELISA and toxin overlay binding competition assays in the presence scFvM22 showed that domain III β16 is involved in the interaction of the pre-pore oligomer with APN. scFvM22 lowered the toxicity of Cry1Ab to M. sexta larvae indicating that interaction with APN is important for in vivo toxicity. Additionally, we show that helix α-3 from domain I contains sequences that could form coiled-coil structures important for oligomerization. Single point mutations in helix α-3 resulted in proteins that bind Bt-

R1 with a similar KD as the Cry1Ab toxin but unable to form oligomeric structures in vitro. These mutants were also severely affected in pore formation and toxicity, indicating that the pre-pore oligomer is an obligate intermediate in the intoxication process of Cry1Ab toxin in insect larvae.

Contributed Paper. Tuesday, 11:30. (55)

Single Mutation in Domain 2 of Cry1Ab Toxin Affects the Insertion of the Toxin into Insect Membranes

Manoj S. Nair1,, Xinyan Sylvia Liu2 and Donald H. Dean1,2*
1Biophysics Program and 2Department of Biochemistry, The Ohio State University, Columbus, Ohio 43210, USA.

The Umbrella and Penknife Models hypothesize that insecticidal Bacillus thuringiensis Cry toxins partition into the apical membrane of the insect midgut by insertion of only two helices from domain I of the protein. Neither model envisages membrane partitioning by domains II and III. In this study, we present data that mutations in domain II residue, F371 affect insertion of the whole toxin into Manduca sexta brush border membrane vesicles (BBMVs). Using steady state fluorescence measurements combined with proteinase K protection assay, we show that mutants of F371 have lost their ability to insert into the BBMV, even though binding to cadherin is almost unaffected. The study also identifies a difference in partitioning of toxins into artificial lipid vesicles (SUVs) as opposed to native BBMVs. While the F371 mutations block insertion of domains I and II into BBMVs, they only block domain II insertion into SUVs. Bioassay and voltage clamping of midguts confirm that the non-inserting mutants are non-toxic. Our study leads us to support models that the toxin enters into the membrane as a whole molecule or oligomers of the toxin. Domain 2 residue F371 has a vital role to play in membrane insertion.

Contributed Paper. Tuesday, 11:45. (56)

GFP expression in wild-type B. thuringiensis strain active to Lepidoptera

Ana Flávia Parente1, José Ivo Baldani2, Ildinete Silva-Pereira1, Victor Hugo da Silva Tibúrcio1, Sônia Nair Báo1, and Marlene T De-Souza1.
1.Cell Biology Department, Brasília University, Brasilia - DF; 2.Embrapa Agrobiology, Seropedica, RJ; Brazil

Although extensively studied by its recognized potential as bioinseticide, B. thuringiensis ecology is poorly understood, and despite its increasing use, little is known regarding interactions between this microorganism and plants. Thus, a tractable marker for detection of this bacterium under specific environment and physiological conditions is a valuable tool. A plasmid (pAD43-25) bearing a functional gfp gene and the parental vector, bearing the promotorless gfp gene, were introduced in the Brazilian wild-type strain B. thuringiensis kurstaki S76, allowing, in the first case, the constitutive synthesis of GFP during vegetative growth. Additionally, both vectors were transferred to a Cry- B. thuringiensis host. Green bright cells could be detected, by fluorescence microscopy and in both hosts, since 2h after inoculation in liquid medium and could be seen throughout remaining cultivation time, until complete sporulation was accomplished. Strain S76GFP+ seems to grow slower than the remaining recombinants and parental cells, whereas no perceptible change in cell or colony morphologies was observed. Protein profile and plasmidial DNA analyses indicate, respectively, that this recombinant maintained Cry proteins expression and resident plasmid outline. To our knowledge, it is the first time that GFP is expressed in wild-type B. thuringiensis strain. Bioassays and sugar-cane inoculation using these cells are under evaluation.

Contributed Paper. Tuesday, 12:00. (57)

Characterization of a new quorum-sensing system in Bacillus thuringiensis

Perchat, S.1, Gominet, M.2., Ramarao, N.1, Nielsen-LeRoux, C.1, Gohar, M.1, Lereclus, D.1
1.Unité Génétique Microbienne et Environnement, INRA, la Minière, 78285 Guyancourt, France.
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During the first stages of stationary phase, B. thuringiensis produces large amounts of extracellular potential virulence factors, including degradative enzymes and toxins. At this stage of development, 80% of the extracellular proteome of bacteria grown in rich medium consist of proteins encoded by PlcR-regulated genes. In absence of PlcR, the extracellular proteome is nearly exclusively composed of two metalloproteases: InhA1 and NprA. It was shown that inhA1 transcription is repressed by AbrB. Bioinformatic analysis reveals that nprA is preceded by two genes encoding a potential regulator (NprR) with a helix-turn-helix domain and tetratricopeptide regions, and a 43-amino acid peptide (NprX) with a potential export signal. This organization is reminiscent of Bacilli genes involved in quorum-sensing. We constructed a B. thuringiensis strain carrying a chromosomal transcriptional fusion between nprA and lacZ, and strains were deleted for nprA, nprR and nprX. The analysis of nprA'-lacZ expression in various genetic backgrounds shows that NprR and NprX are required for nprA transcription. The lack of nprX is complemented by adding, in the growth medium, a synthetic peptide corresponding to the C-terminal end of NprX. Additional complementation experiments indicate that nprA expression depends on a strain-specific quorum-sensing system. The role of NprR/NprA is under investigation.

Contributed Paper. Tuesday, 12:15. (57,5)

Production and characterization of Bt Cry1Ac resistance in cotton bollworm, Helicoverpa zea (Boddie)

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Laboratory-selected Bt-resistant colonies are important tools for understanding possible Bt resistance mechanisms, but some important pest insect species such as the cotton bollworm, Helicoverpa zea (Boddie), have proven difficult to select for stable resistance. Here, laboratory populations of H. zea, resistant to the Bt protein (Cry1Ac) found in all commercial Bt cotton varieties in the US, were established by selection with either activated Cry1Ac toxin (AR) or MVPII(MR). The resistant ratio (RR) for AR reached >100 fold after 11 gen. and has been maintained at this level for 9 gen. since. MR crashed after 11 gen. (similar to previous observations), reaching only a RR of 12 after 7 gen. AR was only partially cross-resistant to MVPII. AR was cross-resistant to Cry1Ab, but was not cross-resistant to other Bt proteins and cypermethrin. Toxin binding assays using 125I-labeled Cry1Aa and Cry1Ac, showed no significant differences between AR and the susceptible strain, indicating that mechanisms of resistance were not linked to a reduction in binding. These results aid in the understanding why this major pest of cotton and corn has not yet evolved Bt resistance, and highlight the need to choose carefully the form of Bt protein used in experimental studies.

CONTRIBUTED PAPERS, Tuesday 10:30 - 12:30

VIRUSES 1. Viral Ecology and Biocontrol

Contributed Paper. Tuesday, 10:30. (58)

Variation in the prey-processing behavior of insectivorous birds affects NPV transmission in the gypsy moth, Lymantria dispar.

James R. Reilly & Ann E. Hajek
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We investigated the potential importance of insectivorous birds in the transmission of gypsy moth nucleopolyhedrovirus (NPV). Bird feeding behavior and the transmission of NPV from infected to healthy larvae were assessed under semi-natural conditions. Three common species of birds that differ in size and are known to feed on gypsy moth larvae were tested: Poecile atricapilla (Black-capped Chickadee), Vireo olivaceus (Red-eyed Vireo), and Dumetella carolinensis (Gray Catbird). We found that these species differed in caterpillar processing behavior and that this variation appears to affect virus transmission. To determine the temporal scale over which birds could be spread the virus, we collected timed fecal samples of birds that had ingested NPV occlusion bodies. We used real-time PCR to quantify the amount of NPV present in the bird feces over time. The effect of gut passage times on transmission will be discussed with respect to bird behavior.

Contributed Paper. Tuesday, 10:45. (59)

Host plant-mediated changes to the peritrophic matrix influence baculoviral pathogenesis

Ruth Plymale1, Diana Cox-Foster2, and Kelli Hoover2
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2 Department of Entomology, The Pennsylvania State University, State College, PA USA

The peritrophic matrix (PM) lines the insect midgut, shielding the midgut epithelium from direct contact with ingested material while permitting passage of nutrients and water. We hypothesized that alteration of the PM in cotton-fed Heliothis virescens larvae may be an important component of the well-documented resistance of cotton-fed H. virescens larvae to nucelopolyhedroviral infection. To test this hypothesis, we monitored larval mortality and in vivo viral pathogenesis in H. virescens larvae fed cotton foliage or artificial diet, using a construct of Autographa californica multiple nucleopolyhedrovirus expressing lacZ under the control of the hsp 70 promoter (AcMNPV-hsp70/lacZ). Significantly fewer cotton-fed larvae displayed lacZ positive infection foci from 12-40 hours post inoculation than larvae fed artificial diet. Further, the PM of cotton-fed larvae was significantly thicker than that of artificial diet-fed larvae. Degradation of the PM by the metalloprotease enhancin reduced PM width in both cotton and artificial diet-fed larvae; moreover, a greater proportion of PM compromised larvae were infected following AcMNPV-hsp70/lacZ inoculation, compared to larvae possessing intact PMs. Our data thus indicates that ingested material can influence PM structure and suggests that food-mediated alterations to the PM may play a role in determining H. virescens larval susceptibility to baculoviral infection.

Contributed Paper. Tuesday, 11:00. (60)

Impact of host plants on the peritrophic matrix as a barrier to baculovirus

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2 Department of Entomology, Penn State University, University Park, PA USA

The peritrophic matrix (PM) lines the insect midgut, providing some protection to the midgut cells and compartmentalizing digestive processes. We hypothesized that alteration of PM structure may be involved in plant-mediated inhibition of mortal infection by Autographa californica M nucleopolyhedrovirus (AcMNPV) in H. virescens. Thus, we compared the PM structure of Heliothis virescens larvae fed either artificial diet or foliage of cotton, tobacco, oakleaf lettuce or iceberg lettuce, and found that the PM of plant-fed larvae was significantly thicker than that of larvae fed artificial diet. Using a construct of AcMNPV expressing lacZ, mean PM width was inversely related to both the proportion of larvae with lacZ signaling in the midgut and/or associated tracheae at 18 hours-post inoculation (hpi) and to final mortality from virus. Also, the proportion of larvae signaling at 18 hpi was positively correlated with the proportion of larvae that ultimately succumbed to infection. Although, the plant-mediated mechanism of inducing a thicker PM is not known, the slower the growth rate of larvae, the thicker their PM. Thus, ingested foliage influences PM structure and this in turn was shown to be an important factor determining the success of baculovirus in foliage-fed hosts.

Contributed Paper. Tuesday, 11:15. (61)

Effects of developmental resistance on LdMNPV pathogenesis in gypsy moth

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Gypsy moth (Lymantria dispar) larvae are differentially susceptible to the baculovirus Lymantria dispar M nucleopolyhedrovirus (LdMNPV) within a larval instar; viral mortality in newly molted larvae is 2-3X higher than in larvae inoculated at 48-72 hours post-molt, a phenomenon called intrastadial developmental resistance (IDR). We hypothesized that IDR is caused in part by immune responses to virus particles or infected tissues, and the extent of immunoresponsiveness is age-dependent within each instar. To test this hypothesis, we orally inoculated 4th instars that had just molted (40's) or were 48 hours post-molt (448's) with a construct of LdMNPV expressing lacZ driven by hsp70. We dissected a subset of larvae daily and processed them for lacZ signaling. We recorded the tissue type(s) signaling lacZ as midgut only, midgut and overlying tracheal elements, tracheae only, and/or systemic infection (other body tissues). We also recorded evidence of cellular immune responses (i.e., encapsulation and/or melanization) on or around the midgut. Susceptible-aged larvae (4o) contained a markedly higher incidence of infected tissues and far less evidence of cellular immune responses than resistant-aged (448) larvae. Also in 448s, the incidence of apparent cellular immune responses increased with time post-inoculation. These findings support the hypothesis that IDR involves anti-viral defenses and that the ability of the insect to mount these defenses is age-dependent within the instar. Interestingly, in larvae inoculated as 40's, the virus appeared to move out of the midgut by two different routes, either via the trachea servicing the midgut or infecting other tissues without infecting tracheae associated with the midgut. Future experiments are planned to determine how gypsy moth recognizes infected tissues as non-self.

Contributed Paper. Tuesday, 11:30. (62)

Inheritance of field resistance of codling moth against Cydia pomonella granulovirus (CpGV)

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The Cydia pomonella granulovirus (CpGV, Baculoviridae) is one of the most important biocontrol agents of the codling moth (CM) in apple production. Since 2003, CM populations with an up to thousand-fold decreased susceptibility to CpGV have been observed in Germany and France. In order to understand this phenomenon of field resistance, investigations on the population genetics and

the mode of inheritance of resistance against CpGV were initiated. These investigations will also provide information about the potential speed of spread of resistance and can help to develop new control strategies or to restore high susceptibility towards CpGV. Mass crossing experiments between a highly susceptible laboratory strain (S) and a resistant codling moth strain (R), which originally descended from a resistant field population, suggested that a dominant factor is involved in the inheritance of resistance. Based on the assumption that the resistant strain was homogeneously resistant we further concluded an autosomal and polygenic inheritance. However, single pair crosses between the two strains S x R indicated that the resistance was not completely fixed in the R strain. Therefore, a new homogeneous resistant codling moth strain was established and the mode of inheritance was re-evaluated by single pair crosses.

Contributed Paper. Tuesday, 11:45. (63)

On the validity of the independent action hypothesis model for the nucleopolyhedroviruses: can infection with a single virion lead to host mortality?

Mark Zwart1,2, Wopke van der Werf3, Felix Bianchi3, Jenny Cory4, Monique van Oers1, Arjan de Visser2, Rolf Hoekstra2, Jan van Lent1, and Just Vlak1.

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3. Crop and Weed Ecology, Wageningen University

4. Department of Biology, Algoma University College, Canada

The 'independent action' hypothesis (IAH) states that a probability of killing a host can be assigned to each individual pathogen entity. It is a straightforward and potentially useful hypothesis for making predictions on the occurrence of co-infections and for pathogen population genetics. This hypothesis has not been tested rigorously in any virus-host system to date. Here, IAH is used as the basis for a numerical simulation to predict the prevalence of co-infections by nucleopolyhedroviruses. As an alternative, a 'group action' model postulates a minimum number of infecting viruses to kill the host. This model predicts a much lower prevalence of single genotype infections than IAH.

To test IAH experimentally, two polyhedrin-positive, bacmid-derived Autographa californica MNPV viruses were generated containing quantitative PCR tags for identification. Polyhedra of both genotypes were used in a 1:1 ratio to challenge Spodoptera exigua L3 and L5 larvae with an LD80 dose. The large number of L3 larvae infected by a single genotype followed the prediction by IAH, thus confirming that a single virion can induce host mortality. In L5 single genotype infections were not found and IAH is rejected. The 'group action' model is more suitable for describing the infection process in final instars.

Contributed Paper. Tuesday, 12:00. (64)

Is there evidence for selection for resistance to viral disease in cyclic populations of tent caterpillars?

Jenny S. Cory 1,2 and Judith H. Myers3

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Epizootics of viral disease are an obvious component of the population fluctuations of several species of forest Lepidoptera. The western tent caterpillar, Malacosoma pluviale, has regular 8 to 11 year population cycles characterised by high levels of mortality from a nucleopolyhedrovirus (NPV) in peak and declining populations. As well as reducing host densities, this high infection has the potential to exert strong selection on the resistance of the host. We examined the variation in larval resistance to NPV in three spatially distinct populations of tent caterpillars, over two years of peak and declining host densities, and one population in year of pre-peak of density. Families of M. pluviale varied greatly in disease resistance in all populations. In year one, one population was significantly more susceptible to NPV than the other three. In the following year, a population that experienced a disease epizootic the previous year was significantly more resistant to virus compared to the other populations. By 2005, following the disease epizootics and initial population declines, it was impossible to accurately bioassay larvae due to their extremely poor survival in the laboratory. All populations declined to very low density by 2006.

Contributed Paper. Tuesday, 12:15. (65)

The use of Baculovirus to control fall armyworm, Spodoptera frugiperda, in Brazil.

Fernando H. Valicente1, Edmar Tuelher2, Rafael. C. Pena2, Renato Andreazza2, Maria R. Fellet 2, Corina, V. Macedo2,4, Ari Gitz 3, José L. C. Wolff.4

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2.Project members financed by FINEP

3.Biocontrole Company

4.Professor – Universidade de Mogi das Cruzes, SP

The Spodoptera fruigiperda nucleopolyhedrovirus (SfMNPV) can be very effective in controlling fall armyworm (S. frugiperda) and has shown potential to be extensively used in Brazil as a biopesticide. However, the widespread application of this baculovirus has been limited mainly due to problems related to its production on a commercial scale. One of the difficulties results from the fact that fall armyworm is cannibalistic. As a consequence, the larvae have to be individualized when used for the multiplication of SfMNPV. This entails increased expenses in materials and labor. Another important difficulty results from the intensive disruption of the larvae integument due to SfMNPV infection. The liquefaction of the integument makes production laborious because all larvae must be frozen before being harvested for polyhedra extraction. Moreover, the liquefaction generally causes significant losses of virus, so additional larvae are needed to produce one dose. An SfMNPV isolate that doesn't disrupt the integument was recently found. The use of this variant allowed us to improve the SfMNPV production system. Our results have showed that between 80 to 120 larvae (from 11 to 13 grams of body weight) are sufficient to produce a dose for one hectare.

CONTRIBUTED PAPERS, Tuesday 10:30 - 12:30

NEMATODES

Contributed Paper. Tuesday, 10:30. (66)

Biochemical and molecular characterization of symbiotic bacteria of four Steinernema from Costa Rica

S. costaricense n.sp.(CR9), S. puntauvense n. sp. (Li6), S. websterii (CR5) and Steinernema sp. (T4) (Rhabditida: Steinernematidae)

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2. Department of Entomology, University of Arizona.

Four Xenorhabdus spp. were extracted from newly recovered Costa Rican Steinernema species: S. websteri (CR5), S. costaricense n. sp. (CR9), S. puntauvense n. sp. (Li6) and Steinernema sp (T4). These four Xenorhabdus isolates were characterized by biochemical, BIOLOG, API 20E, API 20NE, growth temperature and sequence analyses of the 16S rDNA gene. Similarity matrices were calculated using NTSYS-Rohlf (2001). Cluster analyses were performed by UPGMA method. The derived dendrogram based on phenotypic traits placed the four Costa Rican Xenorhabdus isolates into three different clades: CR5 (S. websterii symbiont) was posi-

tioned into one clade near to X. nematophila and the symbionts of S puntauvense and S. costaricense were placed into another clade, but belonging to two different clusters. S. puntauvense symbiont was more closely related to X. bovienii, and the Xenorhabdus sp. associated to S. costaricense was positioned in a separate cluster. Xenorhabdus sp. isolated from Steinernema sp. T4 was placed in a third clade alone. Sequence analyses of 16S rDNA genes confirmed Xenorhabdus CR5 is 96% identical to X. nematophila (RIOBRAVIS), Xenorhabdus Li6 had a 95% of similarity with X. bovienii and CR9 shared 95% of similarity with X szentirmaii and T4 95% with a Xenorhadus sp. strain. Since the similarity results were not conclusive for species identity (less than 97%), further analyses is required to find if the CR5, T4, CR9 and Li6 isolates represent new species of Xenorhabdus genera.

Contributed Paper. Tuesday, 10:45. (67)

A phylogenetic hypothesis on the evolution and interactions of Xenorhabdus spp. and their Steinernema hosts

Ming. M. Lee1, S. Patricia Stock1, Patrick Tailliez2 and Sylvie Pages2

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Entomopathogenic nematodes of the genus Steinernema and their gram negative bacterial symbionts, Xenorhadus spp., are a tractable model system ideal for the study of mutualism. A specialized and intimate relationship exists between nematode and bacteria, affecting many of their life history traits, such as nutrition, dispersal, host-finding, foraging and defense from biotic and abiotic factors. Despite ease of culture in the laboratory and their commercial popularity as biological pest control species, relatively little is known about the evolutionary relationship both within genera and between these two mutualists. In this respect, phylogenetic approaches provide powerful tools for inferring the stability of host–symbiont associations. In this study, we tested the null hypothesis that host and symbiont phylogenetic topologies are congruent. For this purpose, we considered currently available sequence data (28S and 16S rDNA for Steinernema and Xenorhabdus, respectively) to 1.) develop phylogenetic trees for host and symbiont lineages and, 2.) test the hypothesis of co-evolutionary histories and diversification of these two partners. With a thorough understanding of the co-phylogenetic patterns between Steinernema and Xenorhabdus we will be able to hypothesize and make predictions of several genetic, physiological and ecological aspects of this intimate association.

Contributed Paper. Tuesday, 11:00. (68)

Plant lectins showed anti-pinewood nematode activity in vitro

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Lectin proteins were respectively purified from Pinellia ternata and Lycoris radiata. Both P. ternata agglutinin (PTA) and L. radiata agglutinin (LRA) could form polymer in unreductive polyacarylamide gel electrophoresis (PAGE), and could coagulate rabbit red blood cells as well as yeast cells even in a low concentration of 15 μg/ml. Two proteins were respectively diluted to different concentrations such as 500, 250, 125, 62 and 31 μg/ml and then mixed with 100 pinewood nematodes. The mixture was incubated at 25°C, thereafter the nematode survival was measured at 24-hour intervals for total 4 days. Results showed that two lectin proteins both showed significant toxicity to pinewood nematode. More pinewood nematodes were killed when higher concentration of protein was used. Similarly, more pinewood nematodes died when toxic protein acted more longer time to them. Our results indicated that these toxic protein genes could be used as candidates for breeding transgenic pine against pinewood nematode, so our research may highlight a way for using toxic protein gene to breed transgenic plant controlling pine wilt disease in the future.

Keywords: Lycoris radiata agglutinin (LRA); Pinellia ternata agglutinin (PTA); pinewood nematode.

Contributed Paper. Tuesday, 11:15. (69)

Movement of populations of Steinernema carpocapsae relating with searching of mating partners.

Yolanda Reyes Vidal and Mayra de la Torre

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Steinernema infective juveniles males wander large distances searching hosts but the movement behavior during their adult stage is slight known. We found that adult males, put initially on the central point of a pork meat agar slide containing the symbiotic bacteria, dispersed randomly in a larger area (radio, r=2.5 cm) than adult females (r=1.5 cm), after 24 h. However, the male displacement in a mixed population (males and females together) was similar to that of females (r =1.5 cm). Also, we observed that most of the males stop wandering as they reached the females located 1 cm far away at the beginning of the experiment, thus, 47% of males reached and remained together with the females, 28% stayed at the initial position, 13% moved to the opposite direction of the females sector and 12% crossed the female section and went on far away, after 24 h. The male behavior seems to be related with a search for mating. This suggest that chemical signals might be involved.

Contributed Paper. Tuesday, 11:30. (70)

Increased infectivity in Steinernema websteri infective juveniles after development in desiccation stressed hosts

David Easterhoff, Amanda Marion, Becky Reinke, Dr. Susan Bornstein-Forst, Marian College of Fond du Lac

This study investigates the effect of in host desiccation on entomopathogenic nematode (EPN) development and infectivity. Galleria mellonella hosts infected with the EPN Steinernema websteri A10 were allowed to air-desiccate in an environmental chamber set at 230C for up to 31 days post-infection (DPI) resulting in a host weight loss of approximately 64%. Host carcasses were re-hydrated using nonsterile reverse-osmosis (RO) water and placed on 9 mm Whatman filter paper in White traps to collect emergent infective juvenile populations (IJ). Populations were pooled over a three-day time period for time points on days 10, 17, 24, and 31 DPI, respectively. IJ were counted with an apparent peak of approximately 70,000 IJ/host cadavers coinciding with desiccated hosts rehydrated between 17-24 DPI. Desiccation-stressed IJ populations from each time interval were compared with fully hydrated control populations for infectivity using a number of bioassays including lethal time for mortality (LT50), lethal dose for mortality (LD50), number of IJ/cadaver, and sine wave movement. Significant differences ($\alpha<0.5$) were observed for all conditions tested compared with controls. This study has implications for increased infectivity of EPN in field applications.

Key Words: Entomopathogenic nematode, Steinernema websteri, infectivity, dauer larvae, desiccation, stress response

Contributed Paper. Tuesday, 11:45. (71)

Parasitism of Subterranean Termites (Isoptera: Phinotermitidae: Termitidae) by Entomopathogenic Nematodes (Rhabditida: Steinernematidae: Heterorhabditidae)

H. Yu1, D. H. Gouge1, and P. Baker2

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In laboratory bioassays Steinernema riobrave Cabanillas, Poinar and Raulston (355 strain), S. carpocapsae (Weiser) (Mexican 33 strain), S. feltiae (Filipjev) (UK76 strain), and Heterorhabditis bacteriophora Poinar (HP88 strain) were all capable of infecting and killing three termite species, Heterotermes aureus (Snyder), Gnathamitermes perplexus (Banks), and Reticulitermes flavipes (Kollar) in lab sand assays. S. riobrave and S. feltiae caused low levels of Reticulitermes virginicus (Banks), mortality under the same conditions. At 22oC, significant mortality (≥80%) of worker H. aureus and G. perplexus was caused by S. riobrave, in sand assays, indicating the need for further study. Due to the short assay time (3 d maximum) reproduction of the nematodes in the target host species was not recorded. All nematode species were observed to develop to fourth-stage juveniles, pre-adult stages or adults in all termite species with the exception of R. virginicus. Only S. riobrave developed in R. virginicus. Nematode concentration and incubation time had significant effects on the mortality of worker H. aureus. S. riobrave consistently generated the highest infection levels and mortality of H. aureus in sand assays.

Contributed Paper. Tuesday, 12:00. (72)

I know what you have in your stomach: unveiling the secrets of the bacterial vesicle of Steinernema nematodes.

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The intimate association between 3rd stage infective juvenile (IJ) of Steinernema carpocapsae nematode and Xenorhabdus nematophila bacterium has been the main subject in many fields of sciences including symbiosis, biocontrol, pest management, and co-evolutionary biology, mainly due to their exclusive reciprocal mutualism: only X. nematophila can colonize the bacterial vesicle of S. carpocapsae IJ. The nature, origin, and structural integrity of the bacterial vesicle, however, are not well understood. Examination of the vesicle by using transmission electron microscopy (TEM), differential interference contrast (DIC, also known as Nomarski microscopy), epifluorescence, and confocal microscopy has revealed that this bacterial receptacle is not a rigid structure but merely a space defined by two modified intestinal cells. These cells were thought to have microvilli or micro-lamellae with glycocalyx as intestinal cells commonly have. Our preliminary examination shows that some species such as S. feltiae, S. riobrave and S. glaseri possess microvilli or micro-lamellae, whereas others (e.g., S. carpocapsae) do not have such structures. In this presentation we will provide a comparative analysis of the bacterial vesicle at anatomical and ultrastructural levels across representative species of Steinernema taxa. Moreover, a 3-D reconstruction of the bacterial vesicle will be presented and discussed.

Contributed Paper. Tuesday, 12:15. (73)

Do host plant toxins protect Drosophila larvae from wasp parasitism?

Neil Milan, Todd Schlenke. Department of Biology, Emory University, Atlanta, GA.

Drosophila species utilize a variety of host plants, many of which contain toxins. Certain species have evolved resistance to the toxins, and some have even evolved a preference for them. Shifting to a normally toxic host plant may allow these species access to an underutilized food resource or may be a means of escaping parasitism. One possible example of the latter is the rarity with which cactophilic and fungivorous fly species are attacked by parasitic wasps. We set out to test 1) the level of resistance of various wasp parasitoids to toxins naturally found in Drosophila host plants, 2) whether parasitic wasps prefer to attack host larvae grown on standard media versus standard media supplemented with natural plant toxins, 3) and whether wasp eggs are less likely to survive in larval hosts grown on toxic media. The following fly-toxin combinations are being tested: D. melanogaster grown with ethanol, D. sechellia grown with octanoic acid, and D. tripunctata grown with alpha-amanitin. Among our preliminary results, we find that L. boulardi, a specialist parasitoid of D. melanogaster, is more resistant to ethanol knockdown than more generalist parasitoids. Furthermore, L. boulardi females show strong avoidance of D. sechellia larvae grown on media containing octanoic acid.

SYMPOSIUM I, Tuesday 10:30 - 12:30

FUNGI DIVISION: Are Entomopathogenic Fungi only Entomopathogens?

Symposium. Tuesday, 10:30. (74)

Evolution of entomopathogenicity in fungi

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As with all great and complex questions, no definitive answers are possible about the evolution of pathogenicity in general (an eternal question for mycologists!), much less about the evolution of fungal specialization to attack and to kill living insects or other arthropods. It does seem certain, however, that the entomopathogenic habit has arisen multiple times among fungi, and possibly even multiple times within specific fungal groups. It is even possible that some general characters shared by nearly all fungal entomopathogens actually might have played a role in their acquiring that nutritional habit. Speculations about the conditions that allowed a large number and diversity of fungi to become associated with some sucking insects (scales, aphids and cicadas in particular) are plausible but ultimately improvable. And it also seems that such a nutritional habit as entomopathogenicity–and despite any later biological adaptations to arthropod hosts–might be subject to change over time; host switching, even among dramatically different groups of host organisms, may be more common than many might suspect. If life is a banquet, for fungal pathogens it may well be a buffet service in which a preference for any particular food may change and the exploration of new tastes is encouraged.

Symposium. Tuesday, 10:50. (75)

Entomopathogenic fungal endophytes

Fernando E. Vega
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Fungal endophytes are quite common in nature and some of them have been shown to have adverse effects against insects, nematodes, and plant pathogens. We have isolated several fungal endophytes belonging to entomopathogenic genera (e.g., Beauveria, Cladosporium, Clonostachys, and Paecilomyces), but their finding inside plant tissues suggests that they might have a different function in plants. This presentation will provide an introduction to fungal endophytes and will speculate on other possible roles for what insect pathologists traditionally refer to as "entomopathogenic fungi."

Symposium. Tuesday, 11:10. (76)

Beauveria bassiana: endophytic colonization and plant disease control

B.H. Ownley1, M.R. Griffin1, W.E. Klingeman2, K.D. Gwinn1, J.K. Moulton1, and R.M. Pereira3. 1Entomology and Plant Pathology Dept., and 2Plant Sciences Dept., 2431 Joe Johnson Drive, University of Tennessee, Knoxville, TN 37996, and 3Entomology and

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Seed application of Beauveria bassiana 11-98 results in endophytic colonization of tomato and cotton seedlings and protection against plant pathogenic Rhizoctonia solani and Pythium myriotylum. The degree of disease control achieved depends upon the population density of Beauveria conidia on seed and soil type. Using standard plating techniques onto selective medium, endophytic 11-98 was recovered from surface-sterilized roots, stems, and leaves of tomato, cotton, and snap bean seedlings grown from seed treated with 11-98. As the rate of conidia applied to seed increased, the proportion of plant tissues from which 11-98 was recovered increased. For rapid detection of 11-98 in cotton tissues, we developed new ITS primers that produce a PCR product for 11-98, but not for cotton. In samples containing DNA from 11-98 and cotton, the fungus was detected at DNA ratios of 1:1000 (11-98: cotton); 11-98 was detected also in seedlings grown from 11-98 treated seed. Using SEM, hyphae of 11-98 were observed penetrating epithelial cells of cotton and ramifying through palisade parenchyma and mesophyll leaf tissues. Isolate 11-98 induced systemic resistance in cotton against Xanthomonas axonopodis pv. malvacearum (bacterial blight). In parasitism assays, 11-98 hyphae were observed coiling around hyphae of Pythium myriotylum.

Symposium. Tuesday, 11:30. (77)

Lecanicillium spp: aphids and beyond.

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Fungi in the genus Lecanicillium (formerly classified as the single species Verticillium lecanii) are important pathogens of insects and some have been developed as commercial microbial insecticides. In addition, some isolates are hyperparasitic on plant pathogenic fungi, including those that cause powdery mildew. Recently it was demonstrated that several isolates have activity against both aphids and plant pathogens, suggesting the potential of a dual role for Lecanicillium spp. as bioinsecticide and biofungicide. The fungus uses both mechanical forces and hydrolytic enzymes to directly penetrate the aphid integument and the cell wall of the fungal plant pathogen. Activity against Penicillium digitatum is attributed to changes in host cells prior to contact by the Lecanicillium spp. In Pythium ultimatum, in addition to mycoparasitism of the plant pathogen, the mode of action is linked to colonization of host plant tissues, triggering a plant defense reaction. Results of laboratory and greenhouse trials indicate that several species of Lecanicillium have potential to control aphids as well as suppress the growth and spore production of Sphaerotheca fuliginea, the causal agent of cucumber powdery mildew. These results suggest the possibility of dual microbial control of both insect and plant pathogen with one fungal agent.

Symposium. Tuesday, 11:50. (78)

Entomopathogenic fungi and their relationships with the rhizosphere.

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Metarhizium anisopliae is a fungus of considerable metabolic and ecological versatility, being a potent insect pathogen that unusually can also colonize plant roots. This places sharp focus on the biology of the soil root interphase as a site where plants, insects and pathogens will interact. We have employed EST and microarray studies to identify genes underlying ecologically relevant traits and shown that M. anisopliae expresses different subsets of genes on insects and in rhizosperic soils. For example, M. anisopliae adheres to insects and plants using two different proteins, MAD1 and MAD2 that are differentially induced in insects and plant root exudates, respectively, and produce regional localization of adhesive conidial surfaces. Disruption of MAD1 and MAD2 produced approximately 90% reduction in adherence to insects and plants, respectively, suggesting that M. anisopliae conidia possess little if any redundancy of adhesion molecules for the ligands present on plant and insect surfaces. Expression of Mad1 in Saccharomyces cerevisiae allowed this yeast to adhere to insect cuticle. Expression of Mad2 caused yeast cells to adhere to a plant surface. Thus regulation, localization and specificity control the functional distinction between Mad1 and Mad2, and enable M. anisopliae cells to adapt their adhesive properties to different habitats.

WEDNESDAY, AUGUST 15TH

CONTRIBUTED PAPERS, Wednesday 8:00 - 10:00

BACTERIA 2

Contributed Paper. Wednesday, 8:00. (79)

Entomopathogenic bacteria are more virulent than mammalian pathogens to the infection model insect Galleria mellonella

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Galleria mellonnella (Gm) is currently used as a model for identification of virulence factors of mammalian pathogens, but exclusively by injection into the hemocoel. In our laboratory we use Gm to identify chromosomal virulence factors involved in the oral infection process of Bacillus thuringiensis (Bt)/B. cereus (Bc) and particularly those interfering with the intestinal barriers. Indeed, to gain infection in Gm, bacteria are required in association with Cry1C, nor the toxin nor bacteria alone result in larval mortality. This approach has demonstrated the importance of various Bt/Bc virulence factors. In order to evaluate whether Gm could be an oral infection model for mammalian opportunistic pathogens, we have tested strains of B. cereus, B. anthracis(Ba) (attenuated) Enterococcus faecalis (Ef) Listeria monocytogenes (Lm) and Pseudomonas aeroginosa (Pa) and the drosophila pathogen P. entomophila (Pe). 15 Bc strains was screened both as second instars (free ingestion) or in 5th instars (force feeding); all were virulent. Ba, Lm, Ef and Pa strains did not result in larval mortality by force-feeding but Pe was virulent even without toxin. Thus, specific virulence factors adapted to the insect intestine may exist in Bt/Bc and Pe, which are absent from the others. Suggesting a sort of co-evolution between host and pathogen and the links between Bt/Bc.

Contributed Paper. Wednesday, 8:15. (80)

Characterization and role of an iron dependent internalin-like protein expressed during infection.

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Bacillus thuringiensis and Bacillus cereus are closely related gram-positive bacteria with a broad host spectrum. An in vivo expression technology (IVET), promoter–trap system, developed in B. cereus strain ATCC 14579 using an insect host (Galleria mellonella) led to the identification of 20 in vivo induced genes.

A strongly in vivo induced gene designated ilsA was analysed. It encodes an internalin-like protein. As suggested by a Fur box in the promoter region, transcriptional fusion analyses showed expression to be repressed by iron, suggesting that in vivo activation was due to iron deprivation in the host. Transcriptional fusion using the gfp reporter gene indicates that ilsA expression is activated in the hemocoel of the infected larvae, where iron is not accessible to bacteria due to the presence of proteins that scavenge iron. Moreover, disruption of ilsA reduced the bacterial growth rate in iron depleted conditions and reduced the virulence of the bacteria in insects as well as the cytotoxicity in macrophage. These results indicate that IlsA plays an important role in iron acquisition and in the overall pathogenesis of B. cereus. Studies concerning the mechanism whereby IlsA acquires iron from host and identification of the target iron source are currently ongoing.

Contributed Paper. Wednesday, 8:30. (81)

The effects of a bacterial toxin are modulated by the regiolocalization of its specific receptor at the cell surface.

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Bacillus sphaericus binary toxin (Bin) is toxic on larvae of Culex mosquitoes by binding on Cpm1 (Culex pipiens maltase 1) its specific receptor on the epithelial membrane of intestinal cells. We have previously reported that, when expressed in the mammalian epithelial MDCK cell line, Cpm1 (i) is anchored to the plasma membrane by GPI and located in lipid raft microdomains (ii) conserves its ability to bind Bin (iii) treatment with Bin induces the opening of a pore in the cellular membrane and the appearance of intracytoplasmic vacuoles. We report here that when MDCK-Cpm1 cells are treated with nystatin which disrupt lipid rafts Bin induced vacuolation is inhibited. We have generated a mutated Bin receptor, Cpm1mut, aiming to impair the GPI processing. When expressed in MDCK cells, Cpm1mut is targeted to the plasma membrane and promotes Bin specific binding. However Bin does not induce any vacuolation of MDCK-Cpm1mut cells. These results suggest that the mutation generated prevents Bin toxic effect. We have determined Cpm1mut anchoring and partitioning in the plasma membrane to discuss these results as an original strategy to study the involvement of receptor anchored by GPI in lipid rafts in the mode of action of various bacterial toxins.

Contributed Paper. Wednesday, 8:45. (82)

Single point mutations in the Manduca sexta cadherin receptor that affect binding and toxicity of Cry1A toxins

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Bacillus thuringiensis (Bt) produces insecticidal protein toxins during sporulation phase as parasporal crystals. These toxins, called Cry, are highly specific to their target insect, are innocuous to humans, vertebrates and plants, and are completely biodegradable. Therefore, Bt is a viable alternative for the control of insect pests in agriculture and disease vectors of importance in public health. Cry proteins poorly control many insect pests and for many others there are no Cry toxins available for their control. In addition, a major threat for the use of Bt toxins in transgenic plants is the appearance of insect resistance. Therefore of genetic evolution of Cry toxins to kill novel targets or to recover toxicity, in the case of the appearance of resistance in the field, will be needed.

One of the most important molecules in the mode of action of Cry1A toxins is cadherin receptor (CADR), in particular, mutations in insect midgut cadherin proteins that bind Bt toxin are linked with resistance in at least three major lepidopteran pests. It has been demonstrated that a single aminoacid change in the Heliothis virescens cadherin receptor of Cry1A, abolish toxin binding and, most probably, toxicity (1). Binding of Cry1A to CADR facilitates the formation of a pre-pore oligomer that is the toxic form to the insect. Cadherin-like receptor of Manduca sexta is a transmembrane molecule composed of twelve extracellular aminoacid repeats. Repeats 7, 11 and 12 are involved in the binding to Cry1A toxins. In this work we constructed a serial of single point mutants of the CADR repeats 11 and 12. Single point mutations in CADR repeat 12 were sufficient to prevent binding of Cry1Ab and Cry1Ac toxins. Insect bioassays of M. sexta larvae revealed that a CADR fragment corresponding to repeat 12 enhanced Cry1Ab toxicity while fragments that contained the single point mutations had no effect on Cry1Ab toxicity indicating that binding was important for the synergistic effect of fragment 12. To identify Cry1A mutants that recover binding to the CADR mutants, we constructed libraries of variants in the loop 2 and 3 of domain II of Cry1Ac toxin and displayed them on T7 phage as previously reported (2) to perform selection of variants against CADR mutants affected in binding.

References:
(1) Xie, R, M. Zhuang, L. Ross, I. Gómez, D. Oltean, A. Bravo, M. Soberón, and S. Gill. (2005). J. Biol. Chem.. 280:8416-8425.
(2) Sabino Pacheco, Isabel Gómez, Alejandra Bravo and Mario Soberón. (2006). Journal of Invertebrate Pathology. 92, 45-49.

Contributed Paper. Wednesday, 9:00. (83)

Cell-binding and oligomerization of parasporin-2 are mediated by glycosylphosphatidylinositol -anchored proteins.

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Parasporin-2, derived from Bacillus thuringiensis strain A1547, exhibits cytocidal activity against mammalian cells with target specificity. The toxin binds to the toxin-sensitive cells, but not to insensitive cells. During incubation with human hepatocyte cancer (HepG2) cells, the toxin forms SDS-resistant oligomer and then induces permeabilization of plasma membrane. Therefore parasporin-2 seems to be a pore-forming toxin which interacts with putative specific receptors. Here, we report the involvement of glycosylphosphatidylinositol (GPI)-anchored proteins in the cytocidal action of parasporin-2. The treatment of HepG2 cells with phosphatidylinositol-specific phospholipase C, which releases the GPI-anchored proteins from the plasma membrane, reduced the cytotoxicity, cell-binding and the oligomerization of the toxin. In addition, the cytocidal actions were not observed on the GPI-anchored protein-deficient Chinese hamster ovary (CHO) cells. To investigate which GPI-anchored proteins were engaged in the toxin actions, cDNAs of several GPI-anchored proteins were individually transfected into CHO K1 cells and the effects of the toxin were analyzed. As a result, cytocidal actions of the toxin were observed on every GPI-anchored protein expressing cell. These results suggest that the characteristic structure of proteins which are anchored to GPI is required for the cytocidal action of parasporin-2.

Contributed Paper. Wednesday, 9:15. (84)

Role of Cysteine on protein folding and biological activity of the binary toxin "BinB" from Bacillus sphaericus.

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Bacillus sphaericus produce toxin crystal protein which are composed of BinB (51 kDa) and BinA (42 kDa). Both proteins function together to kill mosquito larvae; so called, binary toxin. Since the increasing application of B.sphaericus in the field has recently led to cases of resistance. In order to restrict these resistant development, we need understand the nature and mode of action of B.sphaericus toxins.

It has been proposed that BinB is responsible for the regional binding to the specific receptor while BinA is crucial for the toxicity. The active core of BinB contains three Cysteine residues at positions 67, 161, and 241. In order to investigate the role of Cysteine on the function of the binary toxin, Alanine and Serine substitutions were performed. The results showed that Cys67 and Cys161 of BinB are crucial residues for the toxicity. SDS-PAGE analysis of the wild type and mutant proteins with and without reducing agent showed similar profile. These results suggested that there is no disulfide bond involve in protein folding and inclusion formation. Intrinsic fluorescent spectrum analysis indicated that all mutants should have similar conformation to the wild type protein. The loss of toxicity of Cys67 and Cys161 mutants may due to the loss of interactions between BinA and BinB or BinB and its receptor which are under investigation.

Key words: Bacillus sphaericus, Binary toxin, Cysteine, Disulfide bond, Fluorescent spectroscopy, Toxicity

Contributed Paper. Wednesday, 9:30. (86)

Role of septicemia in the Bacillus thuringiensis mode of action.

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Most of the studies about the Bacillus thuringiensis mode of action have been concentrated on the affinity of δ-endotoxins to membrane receptors and on the pore formation. Subsequent effects on the lost of homeostatic balance, leading to the dead of susceptible insects, has been practically neglected. Previous observations indicate that insects killed by Bt favors bacterial growth, although Bt not necessarily grows profusely in cadavers. Septicemia caused by the invasion of bacteria present in the damaged midgut to hemocoel may be at least one of the direct causes of dead. To obtain evidences on this hypothesis, the strain LBIS-17 of Serratia marcescens was injected into Manduca sexta larvae, causing an almost immediate septicemia; however, when inoculated per os, neither septicemia nor mortality was observed. Then a sublethal dose of the strain HD-73 of B. thuringiensis was fed to M. sexta larvae, alone and along S. marcescens. Mortality of the mixture, evidenced by reddish cadavers, was significantly higher and occurred faster than that caused by the HD-73 acting alone. A subsequent series of experiments and quantitative analyses of data showed that septicemia plays an important role (if not the major role) in the ultimate mode of action of B. thuringiensis.

CONTRIBUTED PAPERS, Wednesday 8:00 - 10:00

FUNGI 2

Contributed Paper. Wednesday, 8:00. (88)

Evaluation of Metarhizium anisopliae morphotypes after UV-radiation and storage

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University of Vermont, USA

Evaluation of Metarhizium anisopliae (Ma) morphotypes after UV-radiation and storage was conducted. The fungal strains P-72 and 85-69p have two morphotypes (Serebrov et al., 2006). Morphotypes P-72-I and 85-69p-I have fluffy colonies, but P-72-II and 85-69p-II have powdery colonies. The insecticidal activity of first morphotypes is higher than second morphotypes. The aim of research was to evaluate four Ma morphotypes for its resistance to UV-radiation and storage. Spore suspensions were treated with UV-radiation for 0.5, 1.0, 2.0, 3.0, 5.0 and 7.0 minutes. The results of UV-radiation tests revealed a sharp decrease in conidia viability of all morphotypes after 5 min of radiation. Therefore, the comparison was made for 0.5-3.0 minutes of the experiment. Ma P-72-1 was the most resistant and 85-69-1 was the least resistant to UV-radiation. The addition of antioxidant to suspension increased the resistance but not to initial value. It is possible that the difference in stability is due to protective action of dark pigment which P-72-1contained, whereas conidia of 85-69-1 are colorless. The liquid and dry formulations of Ma P-72-1 were stored at 5 0C for one year. After each month the viability of spores and biological activity of the morphotype was estimated. The viability of dry conidia and its biological activity was not changed compared with original Ma after one year of storage. However, after 5 months activity of liquid formulation decreased two-fold and the addition of antioxidants provided the conservation of activity partly. We may suggest that differences in virulence between morphotypes formed powdery or fluffy structure was due to different level of enzymatic activity.

Contributed Paper. Wednesday, 8:15. (89)

Hydrophobins and the spore coat of the entomopathogenic fungus Beauveria bassiana

Brett Kirkland and Nemat O. Keyhani
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Phage display was used to clone cDNAs for three hydrophobins from the entomopathogenic fungus, Beauveria bassiana. Sequence analysis of the cloned hydrophobins revealed initiation, coding start, and termination sequences for all three B. bassiana proteins. The proteins encoded by Hyd1, Hyd2, and Hyd3 consisted of 136, 105, and 136 amino acids, respectively. All three proteins contained putative signal peptides, Hyd1 and Hyd2 contained the eight cysteine residues that are a hallmark of hydrophobins, whereas Hyd3 only possessed 5 cysteine residues. The B. bassiana proteins did not display significant homology to each other. The best tBLASTX hits for the proteins were: Hyd1; the G. moniliformis and A. nidulans secreted hydrophobins, Hyd2; the M. grisea and M. anisopliae hydrophobins implicated as virulence factors, and for Hyd3; the ceratoplatanin and Snodprot1 proteins from phytopathogenic fungi. Expression analysis revealed that hyd1 was highly expressed in all samples tested including aerial and submerged conidia, in vitro blastospores, mycelia, and cells sporulating on chitin and insect cuticle. In contrast, hyd2 was not expressed in any of the single cell types, but was constitutively expressed in growing mycelia. N-terminal amino acid sequencing as well as mass spectrometry fingerprinting of a 14-KDa protein found in SDS-insoluble, TFA soluble extracts from aerial conidia identified the major component of the B. bassi-

ana rodlet layer to be the hyd2 gene product.

Contributed Paper. Wednesday, 8:30. (90)

Directed adaptation of entomopathogenic fungi

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As an alternative to chemical pesticides, entomopathogenic fungi such as Beauveria bassiana and Metarhizium anisopliae are currently under intensive study as promising arthropod pest biological controls agents. Strains of these fungi have been selected for control of insects and other arthropods that act as disease vectors including mosquitoes and ticks, crops pests such as whiteflies and borers, and ecologically hazardous, invading pests such as fire ants and termites. Despite their potential several factors have hindered widespread adoption of fungi as part of biological control regimes. Effectiveness under laboratory conditions often does not translate to the high mortality needed for biological control under field conditions. Significant impediments include the relatively low resistance of fungi to abiotic stresses such as solar irradiation and heat. Using a novel automated continuous culture machine that can be used in turbidostat mode and actively selects fast growing variants, M. anisopliae strain 2575 was adapted for growth from 25oC to 40oC. The use of a flexible tubing as growth chamber allows the continuous culture of cells that require a solid support for growth. In principle, this technology can be used to adapt fungal strains to virtually any environmental condition including affecting host range.

Contributed Paper. Wednesday, 8:45. (91)

Process of infection of armoured scale insects (Diaspididae) by the entomopathogenic fungus, Fusarium.

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The fungus Cosmospora (synonym Nectria) (anamorph Fusarium) is a recognised entomopathogen of armoured scale insects. Widespread insect mortality and reductions in pest populations have been commonly observed in the field. Inconsistent mortality rates recorded under laboratory conditions prompted a study into the actual process of infection of armoured scale insects by the fungus. Insect age was a significant factor contributing to mortality, which was much greater when reproductively active insects were infected than when immatures were exposed to the fungus ($P \leq 0.001$).
Examination using scanning electron microscopy found no evidence that the fungus penetrated directly through the protective wax cap of the scale insect or through the adhered interface between the cap and the substrate on which the insect resided. The cap of the reproductively mature insect pulls away from the substrate when releasing crawlers (the mobile pre-settled juveniles), and it was at this time that fungal hyphae were observed growing under the cap. Once the Fusarium hyphae advanced under the cap they readily penetrated the insect body through a number of natural openings (e.g. spiracles, vulva, stylet), with mycosis observed within seven days of infection. Direct penetration through the cuticle of the scale insect was not observed.

Contributed Paper. Wednesday, 9:00. (93)

Avoidance of entomopathogenic strains of Metarhizium anisophliae by termites: An evolutionary perspective

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Termites live in subterranean habitat where they encounter diverse array of microorganisms including entomopathogenic fungus (EPF). The discourse however, has been on how these eusocial insects manage to cope in this hostile habitat. We hypothesized that there is a relationship between pathogenicity and avoidance behaviour. Here we discuss the central role of coevolutionary interactions between isolates of Metarhizium anisopliae and Macrotermes michaelseni. For pathogenicity test, worker termites were inoculated with different concentrations of inoculum. For repellency, worker termites were exposed to different dosages of dry conidia in dual choice olfactometer. The results show correlation between repellency and pathogenicity and stress an insight that pathogenicity and repellency traits have coevolved and have important role in the evolutionary ecology of the termite species. The study epitomizes (i) the most pathogenic isolates require little amount to infect the host; however, they are repellent and (ii) the least pathogenic isolates require substantial amount of inoculum to infect and repel termites, but such amount of inoculum is seldom found in nature. The results of the study also explain, in part, the selective coevolutionary interactions between the termite species and belowground microbial diversity.

Contributed Paper. Wednesday, 9:15. (94)

A study of gene expression of the entomopathogenic fungus Beauveria bassiana on different insect cuticles and synthetic medium through cDNA-AFLP technique

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B. bassiana can adapt to different habits; saprophytic, insect pathogenic and endophytic. The flexibility of B. bassiana with regard to its diet is intriguing. An increased understanding of the molecular mechanisms involved in establishment and development of entomopathogenic fungi on host insects can be regarded as an important step towards their effective use in insect pest management. The gene expression of B. bassiana on cuticular extracts of different insect species (Aphis craccivora, Spodoptera litura, Epilachna vigintioctopunctata and Periplaneta americana) and synthetic medium was analysed. The cDNA-AFLP technique was employed. B. bassiana isolate ITCC 4688, which was found highly virulent on 15 insect species in laboratory bioassays, was chosen for the study. True to its generalist nature, not much difference was observed in the gene expression profiles on cuticular extracts of the tested insects. The few differences observed were in the pathways chosen for regulation of gene expression. In conformation to its generalist nature, B. bassiana exhibited almost a stereotyped programme during its pathogenic phase. No evidence was found in B.bassiana for over production of the multiple types of proteases, lipases and chitinases implicated in breaching the cuticle or utilizing it as a nutrition source that were differentially expressed in the entomopathogenic fungus M. anisopliae.

CONTRIBUTED PAPERS, Wednesday 8:00 - 10:00
VIRUSES 2: Genes and Genomes

Contributed Paper. Wednesday, 8:00. (95)

Ha44 is an essential gene for HearNPV infection and Arg25 is critical for HA44 nuclear localization

Yuan KANG, Fei DENG, Xushi XU, Xiao HAN, Yue JIANG, Hualin WANG and Zhihong HU
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ogy, Wuhan 430071, P.R. China

Open reading frame 44 (Ha44) of Helicoverpa armigera single nucleocapsid nucleopolyhedrovirus (HearNPV) encodes a putative protein of 42 kDa and it is conserved in Group II NPV as well as in GV. Our previous proteomics research has shown that the HA44 is a structure protein of nucleocapsid of HearNPV. To determine whether Ha44 is essential for viral replication, Ha44-deleted bacmid and Ha44-repair bacmid were constructed. Transfection and infection assay demonstrated that the Ha44 is essential for viral replication. Transient expression of HA44 with EGFP fused in frame to the N-terminus showed that the HA44 was localized in nucleus of the cells and appeared as aggregated dots. A series of EGFP fused Ha44 truncations were constructed and transient expression indicated that the nuclear localization signal (NLS) was resided within the N-terminal 130 residues of HA44 and the domain required for aggregation was resided within 187-255 residues of HA44. Bioinformatics analysis suggested that amino acids 24-26, 52-53 were possible NLS of HA44. Plasmids with site mutations of these amino acids were constructed and transient expression assay showed that Arg25 was critical for the nuclear localization of HA44.

Contributed Paper. Wednesday, 8:15. (96)

The role of ME53 in Baculovirus infection

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Baculoviruses encode several immediate early transcripts which include the regulatory genes ie1, ie-0, ie-2, pe38 and me53. Four of these, ie-1, ie-2, ie-0 and pe38 have been studied in detail and were found to play vital roles in viral infection. ORF me53 is expressed at high levels from both early and late promoters in cells infected with Autographa californica multiple nucleopolyhedrovirus (AcMNPV). We generated a me53-null AcMNPV mutant and a repair virus bacmid in E. coli and the infectivity, virus yield and DNA replication of the mutant and repair viruses were compared to the parental wildtype (WT) bacmid. Contrary to recently published results, our data have indicated that Δme53 AcMNPV established virus infection and produced polyhedra in transfected Sf9 and Sf21 cells by 48 hours post-infection. However, Δme53 AcMNPV is severely compromised in the production of the budded virus phenotype and spread of infection to adjacent cells. The repair virus behaved identically to the WT bacmid in all assays, confirming the role of me53 in the mutant phenotype.

Contributed Paper. Wednesday, 8:30. (97)

Characterization of six new Mamestra configurata peritrophic matrix proteins and interaction of MacoNPV enhancin with insect intestinal mucins

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The peritrophic matrix (PM) serves as a barrier to pathogen infection and is composed of chitin and proteins. A viral enhancing metalloprotease, enhancin, has been identified in a number of baculoviruses that increases the oral infectivity of these viruses by degrading select PM proteins. We undertook to identify protein targeted by the Mamestra configurata nucleopolyhedrovirus (MacoNPV) residing within the M. configurata PM. Our tactic was to couple peptide sequence information generated from PM proteins with MALDI-mass spectrometry with expressed sequence tag (EST) data from a midgut-specific cDNA library. Three new insect intestinal mucins (IIM), two small peritrophins with small chitin binding domains and a protein with a chitin deacetylase-like domain were identified. We also demonstrated that feeding of a recombinant AcMNPV expressing the MacoNPV enhancin resulted in increased degradation of McIIM1 relative to the non-recombinant strain. When M. configurata larvae were fed MacoNPV occlusion bodies, we observed that non- and under-glycosylated forms of McIIM1were degraded while the fully glycosylated form of was resistant to degradation.

Contributed Paper. Wednesday, 8:45. (98)

Sequence analysis of a new isolate of Cydia pomonella granulovirus (I12) that breaks CpGV resistance in codling moth

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The Cydia pomonella Granulovirus (CpGV) is one of the most highly pathogenic baculoviruses and an effective control agent of the codling moth (Cydia pomonella), a worldwide pest of apples, pears and walnuts. Three isolates of CpGV were described in the past. The Mexican isolate (CpGV-M) was found in Mexico, whereas CpGV-R was obtained from field collected larvae in Russia and CpGV-E derived from a laboratory strain in England. Analyses of a number of other CpGV isolates originated from Georgia and the Iran indicated that the diversity of CpGV can be explained by three main types of genomes, which we designate as types A, B and C. The A type is predominant in CpGV-M, whereas the B and C types are predominant in CpGV-E and CpGV-R, respectively. An isolate (designated CpGV-I12) containing B type viruses was shown to break the recently observed field resistance of codling moth to CpGV. In order to find the molecular basis of its improved efficacy against CpGV-resistant codling moths, CpGV-I12 was completely sequenced and compared to the genome of CpGV-M1. The sequence comparison will be presented.

Contributed Paper. Wednesday, 9:00. (99)

ORF390 of white spot syndrome virus genome is identified as a novel anti-apoptosis gene

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Actinomycin D could induce apoptosis of shrimp primary cells. However, the apoptosis triggered by actinomycin D was inhibited by WSSV infection. To identify the putative apoptotic suppressor gene of WSSV, overlapping cosmid clones representing the entire WSSV genome were individually cotransfected along with genome DNA of AcMNPVDP35k/pol+. Using this marker rescue assay, a WSSV DNA fragment that was able to rescue AcMNPVDP35k/pol+ infection in Sf9 cells was isolated. By further sequence analysis and rescue assay, the ORF390 was identified as a novel anti-apoptotic gene. The ORF displays two putative caspase9 cleavage sites.The ORF was cloned into the pIE1 vector and then the recombinant vector was transfected into Sf9 cells. The Sf9 cells did not show obvious characteristics of apoptosis when infected with AcMNPVDP35k/pol+. And the transient expression of ORF390 allowed AcMNPVDP35k/pol+ replication in Sf9 cells and resulted in the formation of polyhedra successfully. The results indicate that function of ORF390 in WSSV is a kind of apoptotic suppressor like P35 in AcMNPV.

ORF390 supresses the substrate cleavage of not only upstream initiator capase but also downstream effector caspase, showing that

ORF390 is a broad inhibitor.

Contributed Paper. Wednesday, 9:15. (100)

Gene organization and content of the western tent caterpillar, Malacosoma californicum pluviale nucleopolyhedrovirus

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The western tent caterpillar, Malacosoma californicum pluviale, is a common defoliator of deciduous trees in British Columbia. Tent caterpillars follow a cyclic population pattern every 6-10 years; these cycles are thought to be driven by a baculovirus, Malacosoma californicum pluviale nucleopolyhedrovirus (McplNPV). Genetic variation in McplNPV is widespread in the field population and data on the gene organization and content of McplNPV will provide a basis for studying genetic variation. Analysis suggests that McplNPV is most similar to the group II NPVs. McplNPV has been sequenced, resulting in two contigs with a total of 133,109bp and 128 open reading frames (ORFs) potentially encoding 50 amino acids or more. The G+C content of the genome is 41.6%, while the total area covered by ORFs is 83.3%. Nine homologous regions (hrs) were found with a palindrome similar to other NPV hrs with the highest %id to SeMNPV (51%). McplNPV also has 7 unique ORFs without homologues to other baculovirus. Gene parity plots, phylogenetic trees, gene alignments, and the comparison of McplNPV genome with other baculoviruses will give a better understanding of how the genome functions, and how it relates to other baculoviruses and its host .

Contributed Paper. Wednesday, 9:30. (101)

Genotypic and phenotypic variation of South African isolates of Helicoverpa armigera single nucleocapsid nucleopolyhedrovirus

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Helicoverpa armigera is serious pest of various horticultural and agricultural crops in Africa. Although Helicoverpa armigera single nucleocapsid nucleopolyhedrovirus (HearSNPV) has previously been isolated from South African populations of H. armigera, no studies have been undertaken to evaluate the genotypic and phenotypic variation of South African isolates of this baculovirus. In this study, seven new HearSNPV isolates were obtained from different geographic locations in South Africa. Restriction Fragment Length Polymorphism (RFLP) analysis of viral DNA showed that each one of the new isolates was a genotypically distinct variant. A rapid differentiation method, based on RFLP analysis of virus-specific PCR amplification products, was able to differentiate between each of the new isolates and a reference HearSNPV isolate. Droplet feeding bioassays with neonate larvae showed that the genotypic variants differed significantly in biological activity, with the LD50 value of the least pathogenic genotypic variant being about 100-times higher than that of the most pathogenic genotypic variant.

Contributed Paper. Wednesday, 9:45. (102)

Structural and ultrastructural alterations of Malpighian tubules of Anticarsia gemmatalis larvae infected with different Anticarsia gemmatalis multiple nucleopolyhedrovirus (AgMNPV) recombinant viruses

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Malpighian tubules (Mt) constitute the main excretion organ of insects. Infection by egt- recombinant AcMNPV baculovirus in lepidopteran larvae promotes early degeneration of the Mt, which has been correlated with higher speed of kill. However, no trace of viral infection has been detected in this tissue. We constructed two AgMNPV recombinants with egfp gene under control of hsp70 promoter, being one recombinant egt-, and used another two recombinants (one egt-) containing lacZ gene. Alterations in Mt were analyzed by light and electron microscopies. Bioassays were conducted to compare the recombinants pathogenicity. Results showed progressive presence of marker proteins and tissue degeneration, without traces of infection. Morphological and bioassays results showed increased pathogenicity for lacZ-containing recombinants compared to the egfp ones; and for egt- viruses, we noted higher intensity and anticipation of alterations. Once without infection, we believe Mt degeneration is provoked initially by death of tracheal cells attached to Mt, and later, of Mt cells. Mt cell death might be due to oncosis and apoptosis, which may be activated by depletion of energy reserves and by accumulation of marker proteins, respectively. Absence of egt gene may be leading to higher energetic expense, aggravating Mt cell death, resulting in faster death.

CONTRIBUTED PAPERS, Wednesday 10:30 - 12:30

MICROBIAL CONTROL 2

Contributed Paper. Wednesday, 10:30. (103)

The Effect of Relative Humidity on the Efficacy of Different Isolates of Beauveria bassiana (Balsamo) in Microbial Control of Whitefly, Bemisia tabaci on Cucumber host Plants

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Although Beauveria bassiana is an important agent for management Bemisia tabaci, but humidity is still considered as a valuable factor restricting the use of the entomopathogenic fungi. This study observed the influence of relative humidity on the percentage of mortality accomplished by B. bassiana (Balsamo) Vuillemin on second-instar nymphs of B. tabaci on cucumber plants. Different fungal isolates were used in this trail, fungal suspension of 1× 107 conidia/ ml from each isolate was applied on the target stage of insect. The results showed significantly differences among the isolates under varied regime of %RH. The mortality reached 92.23% at high humidity level achieved by the Bb62 isolate, while the same isolate at the low humidity level caused 73.08% mortality. At high % RH, the % mortality was increased rapidly, and the LT50 value was 4.3 d further a quick suppressing of the pest population was obtained, however, at low %RH, the long-term mortality was achieved with LT50 value of 6.39 d. Thus, the entomopathogenic fungi B. bassiana is a potential agent for microbial control of B. tabaci, with respect to the abiotic factor and particularly humidity.

Key Words: Relative humidity, Beauveria bassiana, Bemisia tabaci, biological control.

Contributed Paper. Wednesday, 10:45. (104)

A simulation model of the greyback cane grub and its pathogens, with special emphasis on Metarhyzium anisopliae

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A simulation model was developed to explore the dynamics of key factors that influence the long-term population dynamics of the greyback cane grub, Dermolepida albohirtum (Waterhouse) (Coleoptera: Scarabaeidae). The model is a stage-specific representation of the cane grub life history. Four years of field data from 2003-2006 suggests that Metarhizium infections may affect greyback cane grub populations in the following year. However, it appears that disease incidence of 25% or greater is necessary for declines in population levels the following year. A statistical disease sub-model was incorporated in a preliminary version of the greyback cane grub model. This model incorporated disease due to the fungus Metarhyzium anisopliae, the protozoan Adelina sp., and the bacteria Bacillus popillae. Simulation runs suggest that disease mortality (from all sources) can significantly regulate cane grub populations, but only in a stochastic version of the model when intra-specific cane grub larval competition was also incorporated into the model. The design of a probabilistic sub-model of Metarhyzium anisopliae based upon encounters with infective cadavers will be discussed.

Contributed Paper. Wednesday, 11:00. (105)

Preliminary results of a field trial using Beauveria brongniartii-conidiospores against the forest cockchafer, Melolontha hippocastani during the main flight in 2006

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In spring 2006, one hundred hectares of forest were treated twice, at intervals of seven days, with an experimental preparation of Beauveria brongniartii-conidiospores during the main flight of the forest cockchafer, Melolontha hippocastani, nearby Darmstadt. Accompanying research was performed by the BBA-Institute for Biological Control and comprised quality control of the experimental preparation, monitoring the fate of B. brongniartii after application, observation of the direct effects on beetles as well as monitoring the effects on the next generation, both in the lab and in the field.

Purity of the conidiospore powder was good, but it contained only half of the anticipated number of viable spores. Thus, only 3.8x1012 spores ha-1 were applied in total. Two days after the second application, up to 4x103 viable spores cm-2 were recovered from leaves. Analysis of soil samples to study the fate of B. brongniartii is in progress. Until the next beetle generation will have emerged in 2010, conclusions on the effect of Beauveria on the cockchafer population will be premature. However, in autumn 2006 less first instar larvae were found in the treated area (24 ± 19 m-2) compared to the untreated control (38 ± 42 m-2).

Contributed Paper. Wednesday, 11:15. (106)

Effects of the Clover Root Weevil Pathogen Beauveria bassiana F418 on Soil Invertebrates and Above Ground Non-target Coleoptera in New Zealand Pastures

Michael Brownbridge, Mark McNeill and Tracey Nelson
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Clover root weevil, Sitona lepidus, is an exotic pest shown to have a severe impact on white clover (Trifolium repens) in New Zealand pastures. Beauveria bassiana F418 strain is being evaluated as a biopesticide for this pest; this development has included an assessment of effects on selected non-target invertebrates.

Soil mesocosm assays were run against the collembolan Folsomia candida, and the earthworm Aporrectodea caliginosa. No adverse effects were observed on F. candida population development, earthworm survival or weight gain over a 12 week study.

F418 was applied at three S. lepidus-infested white clover/ryegrass pastures in October 2006, targeting larvae in the clover root feeding zone.(3-5 cm below ground). Three, 6 weeks, 3 months and 6 months after drilling, sites were sampled for above-ground Coleoptera. Thereafter, insects were maintained in the laboratory to determine the incidence of B. bassiana in individuals that died.

There was site and seasonal variation in the number and diversity of Coleoptera collected. The most abundant were: S. lepidus adults (52%); Argentine stem weevil Listronotus bonariensis, (38%); and Coccinelid spp. (4%). B. bassiana was prevalent at all sites and treatments, and was recovered from all three species. There was a significant site effect on the incidence of B. bassiana amongst beetles but no significant treatment effect.

Contributed Paper. Wednesday, 11:30. (107)

Fungal pathogen for biocontrol of ticks

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Ticks are obligatory bloodsucking arthropods, capable of transmitting diseases in humans, domestic and wild animals, thereby inflicting great economic losses in livestock. On livestock, global losses due to tick and tick-borne diseases (TBD) are estimated at US$13.9-18.7 billion annually, and over 800 million cattle worldwide are constantly exposed. In Africa, they are considered the single most important animal disease problem. In Tanzania alone, total annual national loss due to TBD in 2006 was estimated at US$ 364 million including an estimated mortality of 1.3 million cattle. The use of chemical acaricides remains the main management approach for ticks and TBD; however, their heavy use has resulted in development of resistance to most of the major products. The use of entomopathogenic fungi (EPF) is among the major alternatives being explored. The potential of these pathogens is well documented. Among control strategies, the integration of fungus with semiochemicals is receiving great attention. For instance, members of the Amblyomma genus actively respond to the attraction-aggregation-attachment-pheromones and to CO2 (kairomone) exhale by their host. At icipe, pheromone-baited device that combines EPF, pheromone and CO2 has been developed and field-tested. However, further research is addressing optimization of the attractant device and the entire delivery system.

Contributed Paper. Wednesday, 11:45. (108)

Expressing an insect-specific scorpion neurotoxin makes Metarhizium anisopliae hypervirulent to mosquitoes, beetles and caterpillars.

Chengshu Wang, Monica Pava-Ripoll and Raymond J. St. Leger, Department of Entomology, University of Maryland, College Park, MD 20742

Here we demonstrate a strategy of using the entomopathogenic fungus Metarhizium anisopliae to deliver a specific toxin, in a tissue specific manner without compromising host-specificity or contaminating the environment by production during saprophytic growth. We first identified the promoter of the M. anisopliae collagen-like (Mcl1) gene as specifically targeting expression to the host hemocoel. We then expressed a synthetic gene encoding an insect-specific neurotoxin (AaIT) from the scorpion Androctonus australis in a strain of M. anisopliae pathogenic to some lepidopterans, coleopterans and dipterans. Virulence was increased 22-fold against tobacco hornworm (Manduca sexta) caterpillars, 14-fold against Broca (Hypothemenus hampei) beetles and nine-fold against adult yellow fever mosquitoes, Aedes aegypti, with pre-lethal effects including reduced mobility and feeding. Employing green fluorescent conidia we demonstrated that fewer than four recombinant spores are required to kill a mosquito. However, specificity was retained as the recombinant strain did not cause disease in non-host species for the parental wild type strain. This study demonstrates that genetic engineering can produce new levels of virulence in a commonly used pathogenic fungus, and potentially extends the deployment of

the very useful, well characterized AaIT beyond the lepidopterans principally targeted by baculoviruses.

Contributed Paper. Wednesday, 12:00. (109)

Whey-based fungal micro-factories for in situ production of biological control fungi

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Whey-based fungal micro-factory technology is being developed to overcome economic and physical constraints associated with use of mycopathogens for management of insects and other pests. This novel formulation technology is based on inclusion of sweet whey as a nutritive substrate to allow bio-control fungi to grow and multiply post-application. Laboratory experiments using the entomopathogen Lecanicillium muscarium applied to either sterile Petri dishes or field collected hemlock foliage produced dramatic increases in number of entomopathogen spores, as high a 200-fold. Extensive fungal growth occurred on treated hemlock foliage. Lecanicillium muscarium has activity against hemlock woolly adelgid (Adelges tsugae), an invasive pest causing serious damage to the eastern hemlock and Carolina hemlock. The dramatic increases in spore concentration and the fungal ability to out compete resident epiphytes on foliage for the whey resource highlights the potential for whey-based fungal micro-factories as a simple and inexpensive technology for increasing post-application abundance of bio-control fungi. Micro-factory production was inhibited at high initial spore concentrations, indicating that formulations must be optimized for maximal in situ production. This technology is likely applicable to fungal bio-control agents other than entomopathogens (insect-killing fungi), such as, mycoherbicides, and fungi for management of mites, diseases, and nematodes.

Contributed Paper. Wednesday, 12:15. (110)

Impact of a treatment of Beauveria bassiana (Deuteromycota: Hyphomycetes) on honeybee (Hymenoptera: Apidae) colony health and on varroa mites (Acari: Varroidae)

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In spring 2005 the insect pathology team at the European Biological Control Laboratory found entomopathogenic fungi, Beauveria bassiana, on varroa mites from beehives in the Languedoc-Roussillon region of southern France. Strains of the fungus were tested in laboratory bioassays, and the fungi were found to kill the mites in a median of 5-8 days. Two small field experiments were conducted in the fall of 2005, during which the fungal treatment, consisting of B. bassiana conidia formulated with wax powder, was found to cause a significantly higher mite fall than untreated hives. A third field experiment was conducted in spring 2006 using 21 hives, primarily to determine whether the fungus, which can attack bees, caused any health problems for beehives. Treatment of hives with the fungus caused signficantly higher mite fall, but did not cause any measurable negative impact on hive health. Preliminary results on a field experiment involving different kinds of wax powders and more than one treatment will also be discussed. Future experiments are planned with the intention of getting a reasonably inexpensive product on the market in the near future.

Keywords: Apis mellifera, Varroa destructor, Beauveria bassiana, biopesticide, formulation

IFENSB Session III, Wednesday 10:30 - 12:30

INFECTION & STRESS BIOLOGY

Symposium. Wednesday, 10:30. (111)

Entomopathogenic Nematodes from Xerophitic Habitats: Diversity, Distributional Patterns and Ecological Notes [EPN FORUM-Stress Biology and Ecology Session]

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Degradation of soils and overuse of the land is a topic of particular concern in desert or semi-desert ecosystems, where rational management requires increased knowledge of the ecology of these soils, including knowledge of the diversity of the soil flora and fauna and their respective interactions. Nematodes and insects are among the most abundant faunal components of the soil. Several studies have shown the significance of nematodes as below-ground herbivores, in decomposition and mineralization processes, and as natural control agents of soil-dwelling insect pests and plant-parasitic nematodes. In this last category, insect-parasitic and pathogenic nematodes (EPN) are the most widely known and studied group of invertebrate parasites. Thus far, several EPN species have been recovered from arid and semi-arid ecosystems, mainly from Asia and North America. These new species/isolates represent new germplasm that may offer more suitable alternatives for control against a variety of pests in xerophytic agriculture. In this presentation, I will review the diversity and distribution of EPN in desert and semi-desert habitat. Emphasis will be placed on a recent study conducted in SW Arizona. Ecological traits and control potential of these native EPN in arid and/or semi-arid regions will be presented and discussed.

Symposium. Wednesday, 10:55. (112)

Low Temperature Infection and Survival Strategies of Entomopathogenic Nematodes

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Low temperature can have a profound impact on the infection dynamics and survival of entomopathogenic nematodes. The non-feeding developmentally arrested infective juvenile is the most important survival stage both within the host and in the soil. Infective juveniles may survive low temperature using several strategies, including freeze avoidance, freezing tolerance and latent infection. Low temperature can also compromise infective juvenile ability to penetrate and successfully establish within a host. Within the host cadaver adults, other larval stages and eggs can also survive low temperature and freezing. To date, most low temperature survival studies have been conducted under laboratory conditions and the fate of over wintering nematodes in extreme soil environments is therefore largely an extrapolation of these findings. A review of nematode low temperature survival strategies is made to further elucidate the fate of entomopathogenic nematodes at low temperatures in the external environment.

Symposium. Wednesday, 11:20. (113)

Trait modification in entomopathogenic nematodes

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A number of beneficial traits such as virulence, reproductive potential, and environmental tolerance are key factors in determining an organism's ability to produce high levels of efficacy in biological control. Beneficial traits in entomopathogenic nematodes have been enhanced through molecular methods as well as non-molecular methods including selection, hybridization, and bacterial transfer. Deterioration or loss of beneficial traits during laboratory or industrial culture production is detrimental to biocontrol efficacy. During in vivo production, both partners in the nematode-bacterium complex can undergo change, which contributes to reduction in beneficial traits. The nematode's bacterial symbiont also deteriorates when repeatedly cultured in vitro. Trait deterioration can be deterred through creation of selected inbred lines or improved cryopreservation techniques.

Symposium. Wednesday, 11:45. (114)

Sex-specific dispersal and infection in Steinernema spp

Christine Griffin, Department of Biology, National University of Ireland, Maynooth, Ireland.

Most species of Steinernema reproduce by amphimixis and the sex ratio is approximately balanced. The mating success of adult worms depends to large extent on their prior behaviour as immature infective juveniles. There is evidence that infective juveniles of entomopathogenic nematodes have complex infection strategies, and it has been proposed that the strategies of those juveniles that will develop into males or females may differ. Amongst the hypotheses that have been proposed are the "male colonisation" hypothesis which holds that male infective juveniles of some Steinernema species are attracted to and invade distant hosts before females, and the related "protandrous male" hypothesis which holds that male infective juveniles of cruise-foraging species emerge from the natal host earlier, and are more responsive to volatile host stimuli, than females. I will review the evidence that infective juveniles that will develop into males and females differ in their behaviour.

Contributed Papers, Wednesday 10:30 - 12:30

Viruses 3: Molecular Aspects of Virus-host Interaction

Symposium. Wednesday, 10:30. (115)

Transcriptomics of the baculovirus Choristoneura fumiferana multicapsid nucleopolyhedrovirus (CfMNPV)

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Choristoneura fumiferana multicapsid nucleopolyhedrovirus (CfMNPV) is in the Baculoviridae family. CfMNPV is pathogenic to the eastern spruce budworm, C. fumiferana, which is historically the most destructive pest of fir and spruce forests in North America. The CfMNPV 129 593 bp long double stranded DNA genome encodes 146 computational open reading frames (ORFs). The transcription pattern and the functions of the majority of the CfMNPV genes have not yet been described. In this study, we begin to temporally define the entire CfMNPV transcriptome using an oligonucleotide based two-channel DNA microarray. We developed a novel normalization protocol using CfMNPV viral genomic DNA (vgDNA) as equimolar reference standards. A microarray chip was constructed containing oligonucleotide probes for the 146 CfMNPV ORFs and their complements. To follow the temporal expression pattern of these genes and their complements, Cf203 insect cells were infected with CfMNPV at a multiplicity of infection (MOI) of 10 and total RNA was isolated at various times post infection. The cDNA was synthesized, fluorescently labelled with Cy3, and co-hybridized to the microarray chips with Cy5-labelled vgDNA. From the initial microarray data the temporal gene expression profiles could readily be classified into several distinct categories.

Symposium. Wednesday, 10:45. (116)

Reprogramming the Autographa californica multiple nucleopolyhedrovirus chitinase expression profile

Jeff Hodgson 1, Basil Arif 2, and Peter Krell 1
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Reprogramming for increased expression of baculovirus chitinase (and putatively cathepsin) expression using native baculovirus promoters might provide means for designing environmentally benign biological insecticides. Expressions of chiA and v-cath RNA and enzyme activity in infected Sf21 cells were compared with that of recombinant viruses reprogrammed for expression of the endogenous chiA. To establish a baseline for our recombinant AcMNPV studies, we monitored temporal v-cath transcription and compared, for the first time, the temporal expression profiles of both chiA transcription and translation simultaneously. Replacing 21 nucleotides containing the native late promoters with a selectable polh-EGFP cassette was sufficient to abrogate both chiA and v-cath expression. Exchanging EGFP with either the p6.9 or polh promoters to drive chiA transcription produced marked differences in the temporal chiA transcription profiles relative to that from the native promoter. Furthermore, when chiA was transcribed from the native, p6.9, or polh promoter, the specific activities of chitinase at 48 h.p.i. were 62, 160 and 219 mU/mg of the lysate total protein, respectively. Transcription of v-cath was detectable for AcMNPV by 9 h.p.i., but v-cath RNA or enzyme expression was undetectable through 48 h.p.i. in the chiA-reprogrammed viruses bearing the reconstituted v-cath promoter.

Symposium. Wednesday, 11:00. (117)

Escape mutants of Autographa californica multiple nucleopolyhedrovirus (AcMNPV) resistant to nucleoside analogues

David K. Thumbi 1, Basil M. Arif 2 and Peter J. Krell1 1 Department of Molecular and Cellular Biology, University of Guelph, ON, N1G 2W1 2 Great Lakes Forestry Centre, Sault Ste Marie, ON, P6A 2E5

The Autographa californica multiple nucleopolyhedrovirus (AcMNPV), is a prototype of the family Baculoviridae, which encompasses entomopathogenic viruses currently being used as biocontrol agents against major pest in agriculture and forestry. Little is known about their molecular mechanisms of DNA replication. In this study we generated and characterized two AcMNPV drug resistant mutants. Since the nucleoside analogues aphidicolin (APC) and abacavir (ABC) are known inhibitors of viral DNA polymerases, we hypothesized that "escape" mutants derived from treatment of the parental AcMNPV with these drugs would harbour genomic changes in the substrate binding sites of the AcMNPV DNA polymerase. Two mutants, AcAPCr and AcABCr, were generated by serial passage of the parental AcMNPV in the presence of increasing concentrations of APC and ABC, respectively. Preliminary results showed the mutants had similar in vitro growth properties as the parental virus. Sequence analysis of AcAPCr and AcABCr DNA polymerases revealed mutations within the conserved regions III (S-611-T) and II (C-543-R), respectively. The results of this study may help reveal the functional nature of baculovirus DNA polymerase in

DNA replication.

Symposium. Wednesday, 11:15. (118)

Functional analysis of a putative inhibitor of apoptosis (IAP) encoded by Chilo iridescent virus

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Programmed cell death or apoptosis is a major defense mechanism used by insects in response to viral infections. The viruses can only successfully infect the host if they inhibit this apoptosis. The genome of Chilo iridescent virus (CIV) has three ORFs with homology to baculovirus inhibitor of apoptosis (iap) genes. The proteins encoded by the 157L, 193R, and 332L genes contain 152, 208 and 234 amino acids, respectively, and have 23–41% similarity to the IAP-3 protein of Cydia pomonella granulovirus. The protein encoded by 193R contains a BIR motif (baculoviral iap repeat) in the amino-terminal segment and a carboxy-terminal RING finger motif. The presence of a BIR domain in a CIV open reading frame is a unique feature among Iridoviridae. RT-PCR showed that 193R is transcribed in a CIV infection. When this putative CIV iap was transiently expressed in SPC-BM-36, Sf21 or TniH5 cells under control of an early baculovirus promoter it blocked apoptosis induced by actinomycin-D. RNA interference was used to knockdown the expression of 193R during CIV infection in SPC-BM-36 cells. The conclusion from these results is that CIV ORF193R encodes a functional IAP protein.

Symposium. Wednesday, 11:30. (119)

Deletion within the AcMNPV IE0 N-terminus 54 amino acids reduces its ability to support viral DNA replication

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AcMNPV IE0 and IE1 have been showed to support virus growth and both are required to obtain a wild type infection. They have also been shown to differentially activate, and are mutually antagonistic for late gene expression. IE0 and IE1 therefore appear to have very different regulatory roles in the viral infection cycle even though AcMNPV IE0 differs from IE1 by only an additional 54 amino acids (aa) at the N-terminus. How this extra 54 aa alters the function of IE0 from IE1 during infection remains unknown. In this study a mutational analysis of the 54 aa IE0 specific N-terminal domain was performed. Deletion of IE0 amino acids 2-43 and 39-54 showed reduced DNA replication before 36 hpi but similar levels as full length IE0 were obtained by very late times post infection. Transmission electron microscopy was used to see if there is any difference in the phenotype of nucleocapsids and polyhedra in the cells infected by viruses expressing only IE0 or IE1. To further elucidate the function of IE0 and IE1 we used tandem affinity purification and LC-MS/MS to identify interacting proteins.

Symposium. Wednesday, 11:45. (120)

The baculovirus occlusion-derived virus envelope protein P74 requires site-specific cleavage by insect midgut trypsins for function in per os infection.

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Baculoviruses have been used for decades in biocontrol of insects. Insects become vulnerable to infection when they ingest foliage contaminated with viral occlusion bodies. Liberated occlusion-derived virions initiate infection in epithelial cells when OBs dissolve in the alkaline environment of the larval midgut. It has been shown that P74, present on the surfaces of ODVs, was essential for infection. We have observed that P74 of the Autographa californica (Ac)MNPV is N-terminally cleaved when incubated with insect midgut tissues under alkaline conditions and that cleavage was prevented by soybean trypsin inhibitor (SBTI). We made a series of P74 mutants which lacked specific trypsin cleavage sites. Removal of the trypsin cleavage sites R195, R196 and R199 in P74 compromised per os infectivity. Specific trypsin cleavage of P74 by insect midgut trypsins was lost after this mutagenesis. A series of N-terminal and C-terminal deletions of P74 revealed a core functional domain. Most significant was the discovery that the C-terminal transmembrane anchor domain of P74 was not required for function.

Symposium. Wednesday, 12:00. (121)

Functional analysis of HearNPV putative anti-apoptotic genes

Changyong Liang1. 2; Xinwen Chen1; Monique van Oers2; Just M.Vlak2; Marcel Westenberg2.
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Baculovirus infection induces an apoptotic response in cultured insect cells, which can severely limit viral replication. To overcome this host response, all baculoviruses studied to date carry anti-apoptotic genes, including members of the p35 and iap (inhibitor of apoptosis) gene families. The baculovirus Helicoverpa armigera nucleopolyhedrovirus (HearNPV) carries two putative apoptosis suppressor genes: iap-2 and iap-3. IAPs are functional in the cytoplasm, but surprisingly, transiently expressed IAP-2 was evenly distributed throughout the cell, while IAP-3 was mainly found in the nucleus. When the cells were infected with HearNPV, IAP-3 was also evenly distributed. HearNPV with an iap-2 deletion could still be propagated in Hz2e5 cells, while an iap-3 deletion was lethal. The HearNPV Δiap-3 mutant could be rescued by AcMNPV p35 or OpMNPV iap-3, but not by HearNPV iap-3 when driven by the OpMNPV ie-1 promoter. Reinsertion of HearNPV iap-3 driven by its own promoter rescued virus replication, indicating that the timing of IAP3 expression is crucial. Furthermore, RNAi analysis showed that HearNPV induced apoptosis in Hz2e5 cells transfected with iap-3 dsRNA, while silencing of iap-2 did not result in apoptosis. These results together indicate that HearNPV IAP-3 is a functional apoptosis suppressor, while the function of IAP-2 remains elusive.

Symposium. Wednesday, 12:15. (122)

Baculovirus infection of an insect host immunosuppressed with cys-motif and vankyrin polydnavirus genes

Nor Chejanovsky1, Haddassah Rivkin1, Irit Ornan1, Bruce A. Webb2
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2Department of Entomology, University of Kentucky, Lexington, KY 40546-0091,USA

The Mediterranean pest Spodoptera littoralis is highly resistant to infection by the baculovirus AcMNPV. We have previously demonstrated that polydnavirus-mediated immunosuppression of S. littoralis resulted in enhanced susceptibility to AcMNPV via intrahemocoelic infection. To further study the effect of immunosuppression on the above host-baculovirus system using specific polydnavirus

genes, we constructed polyhedra-positive recombinant baculoviruses bearing polydnavirus genes belonging to the cys-motif and vankyrin families. Moreover, we followed the path of infection with GFP-tagged recombinant baculoviruses bearing those polydnavirus genes. This approach resulted in significant enhancement of AcMNPV oral infectivity to S. littoralis and provided some more hints about the AcMNPV path in the immunosuppressed host.

STUDENT COMMITTEE SESSION ,Wednesday 12:00 to 14:00
With pizza lunch

Student Session. Wednesday, 12:00. (123)

Skills in Getting a Position in Private Industry

Ramon Georgis
Valent Biosciences Corporation, Libertyville, IL, USA

Agrochemical and agbiotechnology companies offers attractive and career opportunities in various disciplines of agriculture and biological sciences. Although academic performance is of important, equally these companies are looking for candidates with high communication skills, ability to work in complex projects that involved employees with different disciplines and skills, and the ability to become a leader by demonstrating high performance and effective dialogs with co-workers.

Student Session. Wednesday, 12:30. (124)

Academic interviews: get one step ahead

Bryony C. Bonning, Department of Entomology, Iowa State University, Ames, IA, USA

It is not uncommon for applicants for academic positions to be ranked first prior to an interview and to be demoted as a direct result of their performance at the interview. This talk will highlight strategies for getting one step ahead, and indicate how you can improve your chances of being offered the position. The content will be based in part on my own experience as an interview candidate, and as a faculty member at Iowa State University assessing interview candidates. I will review interview format, questions that applicants should be prepared to ask and to answer, preparative research on the institution and faculty, the research seminar and fielding of questions, teaching seminar and philosophy, “talking turkey” with the chair person, and the art of negotiation.

Student Session. Wednesday, 13:00. (125)

The road from PhD to tenure track faculty

Juan Luis Jurat-Fuentes1
1Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN 37996

Now that you have your PhD, what’s next? Would you want a position in industry or in academia? Should you start looking for a postdoc? How do you prepare an interview? Decisions taken after graduation will greatly affect your future scientific career. In this presentation the speaker will share his personal experiences and dilemmas in obtaining an academic tenure-track position.

Student Session. Wednesday, 13:30. (126)

Job hunting strategies for government employment

Fernando E. Vega

CROSS-DIVISIONAL SYMPOSIUM, Wednesday 14:00 - 16:00
Advances in the use of Microbial Agents for Control of Orchard Pests

Symposium. Wednesday, 14:00. (127)

Codling moth granulovirus: Learning from Europe while defining use strategies for North American orchardists

Donald R. Thomson1, Lawrence A. Lacey2, Robert Fritts Jr. 3, Larry J. Gut4 and Charles Vincent5
(1DJS Consulting Services, Seattle, WA 98146, USA; 2USDA ARS, Yakima, WA 98951, USA; 3Advan LLC, Clovis, CA 93619, USA; 4Michigan State University, East Lansing, MI 48424, USA; 5Agriculture and Agri-Food Canada, Saint-Jean-sur-Richelieu, Quebec, J3B 3E6, Canada)

The granulovirus of codling moth (CpGV), one of the most virulent baculoviruses, was first isolated from infected larvae in Mexico in 1963. Following extensive research conducted both in Europe and North America, CpGV is now used on over 100,000 hectares annually in Europe, but its adoption is only beginning in North America. Benefits in codling moth pest management include its efficacy, selectivity, safety to applicators and the food supply and minimal re-entry and pre-harvest intervals. Limitations include solar degradation resulting in short residual activity, slow speed of kill resulting in cosmetic damage to fruit and cost of product. This presentation examines the differences between the North American and European experiences with CpGV with respect to its adoption and fit in codling moth pest management programs. What were factors that drove the adoption of CpGV in Europe but limited its use in North America? The presentation will identify recent research conducted or underway to advance the adoption of CpGV in North American orchards. The presentation will also identify the barriers and impediments that limit more extensive use. Suggested research to help overcome these hurdles will also be discussed.

Symposium. Wednesday, 14:15. (128)

Field resistance to Cydia pomonella granulovirus - a new challenge for the biological control of codling moth

J. A. Jehle
Laboratory of Biotechnological Crop Protection, Department of Phytopathology, Agricultural Service Center Palatinate (DLR Rheinpfalz), Neustadt a. d. Wstr., Germany

Cydia pomonella granulovirus (CpGV) products have obtained great importance in codling moth control in both organic and integrated apple production. Their application is estimated to be about 150 000 ha world wide per year. Since 2003, local field populations of codling moth, which show an up to 1000fold reduced susceptibility to CpGV, have been observed in Germany, France and a few other countries in Europe. A spread of this phenomenon is a severe threat to the efficient control of the codling moth, particularly in organic farming. Recently, more information on the geographic distribution, the mechanism and the mode of inheritance of the resistance became available. In addition, new CpGV isolates, which are able to overcome the resistance, were isolated. These isolates have very promising features in bioassays as well as field tests and might be used in the future to improve the control of the resistant codling moth populations. The presentation will give an overview on recent developments as well as different lines of research in Europe.

Symposium. Wednesday, 14:30. (129)

Entomopathogenic nematodes for control of codling moth in pome fruit

Lawrence A. Lacey
Yakima Agricultural Research Laboratory, USDA-ARS, 5230 Konnowac Pass Road,
Wapato, WA 98951, USA

Apple is grown as a crop in temperate zones throughout the world and codling moth (CM) is one of its most serious pests. Control of overwintering CM larvae would reduce or eliminate damage to fruit early in the following growing season. Entomopathogenic nematodes (EPNs) have shown promise as biological control agents of cocooned CM larvae in cryptic habitats in California and Pacific Northwest orchards. Field trials with Steinernema carpocapsae and S. feltiae were conducted in apple and pear orchards to determine

the effects of seasonal temperatures, post-application irrigation and method of application on larval mortality. Trials in late summer, early fall and late spring, using 106 infective juveniles (IJs)/tree plus supplemental wetting to aid survival of IJs produced 94-95% mortality in sentinel CM larvae when mean temperatures were above 20°C. When temperatures dropped to a mean of 12-13°C, control by S. feltiae was still effective (90% mortality). Application with an airblast sprayer was as effective as treatment of trees with a hand held sprayer when orchards were kept wet for several hours. Wood chip mulch enhanced control by providing cocooning sites for CM larvae and a substrate that maintained moisture.

Symposium. Wednesday, 14:45. (130)

Use of Bacillus thuringiensis for the control of Lepidopteran pests in apple orchards.

Jean-Charles Côté
Horticulture Research and Development Centre, Agriculture and Agri-Food Canada, Saint-Jean-sur-Richelieu, QC, Canada

The bacterium Bacillus thuringiensis (Bt) was initially isolated from dead silkworm larvae a century ago. This initial discovery would direct researches on Bt for the next 100 years. It has successfully been developed and used on cole crops, in orchards and in forestry for the control of Lepidopteran pests. It has been used for the control of several other insect pests and some other invertebrates in various environments.

In collaboration with the private sector, we have contributed to the development of some Bt-based formulations and their registrations, including one, Bioprotec CAF, for use in apple orchards. This formulation, like other Bt var. kurstaki-based formulations, is highly toxic against selected insect pests, here tortricid leafrollers. These formulations are, in general, also compatible with parasitoids and predators. They are not, however, efficient against another economically important orchard insect pest, the codling moth. The efficacy of many of these formulations rapidly decreases over time loosing 50% of their activity within 24 h. An improved Bt formulation, Bioprotect 3P, has also been developed with better resistance to UV rays and rain and extended efficacy in the field.

Interestingly enough, despite the development and formulation of a few strains into bacterial insecticides, most Bt strains do not exhibit known insecticidal activities. Questions will be raised regarding the true role of Bt in the environment.

Symposium. Wednesday, 15:00. (131)

Developing an IPM system with entomopathogenic nematodes for guava in Brazil

C. DOLINSKI Universidade Estadual do Norte Fluminense Darcy Ribeiro/CCTA/LEF. Av. Alberto Lamego, 2000, Campos dos Goytacazes, RJ, Brazil. 28015-620. claudia.dolinski@censanet.com.br

Guava is becoming one of the most important fruit crops in the State of Rio de Janeiro, Brazil. Our light soils, abundant water and sunny weather are perfect for guava production. The down side is the presence of pests and diseases affecting fruit quality. The guava weevil (Conotrachelus psidii) is considered a major pest because it directly damages the fruit and is difficult to control with chemical pesticides. Weevil adults appear in September-October and remain in the field until March. The 4th instar larvae are present in the soil and pupate after a variable time interval. In association with guava growers from Cachoeiras de Macacu-RJ, we are developing an integrated pest management (IPM) program for this pest using entomopathogenic nematodes (EPNs) as biological control, plus cultural control and alternative chemical control. For EPN application, we are testing different methods including infected insect-cadaver, spray system and agar-water suspension. All methods were tested with Heterorhabditis baujardi LPP7 with great success under lab and field conditions. Now growers can choose from these different methods, which one is more feasible to their reality.

Symposium. Wednesday, 15:15. (132)

Microbial control in Florida citrus groves: problems, perils, and potential for enhancing the effectiveness of entomopathogenic nematodes for root weevil control

Robin J. Stuart and Larry W. Duncan
University of Florida, IFAS, Citrus Research and Education Center, Lake Alfred, FL, 33850 USA

The use of entomopathogenic nematodes (epns) to control root weevils in Florida citrus groves is considered a biological control success story. Experiments in the laboratory, greenhouse and field have demonstrated that the nematodes can kill weevils and increase yield, and commercial nematode products have been successfully marketed for weevil control in Florida citrus for over 15 years. But can we improve on this success? Recent research has shown that epn applications are much more effective in groves grown on certain kinds of soils than on others, and that groves where nematode applications are least effective often have especially sparse endemic faunas of epns and serious weevil problems. Current research is directed toward developing epn augmentation strategies that can be effective on different soil types and in exploring the potential of inoculative and conservation biological control strategies.

Symposium. Wednesday, 15:30. (133)

Microbial control of plum curculio and peachtree borers

David Shapiro-Ilan1, Ted Cottrell1, Russ Mizell2, and Dan Horton3
1USDA-ARS, SE Fruit and Tree Nut Research Lab Byron, GA 31008
2Department of Entomology, University of Florida, Quincy, FL
3Department of Entomology, University of Georgia, Athens, GA

Plum curculio, Conotrachelus nenuphar, is a major pest of stone and pome fruits. Stone fruits are also plagued by clear-winged moths (Lepidoptera: Sesiidae), e.g., peachtree borer (Synanthedon exitiosa) and lesser peachtree borer (Synanthedon pictipes). Microbial control agents have potential as alternative management tactics for these pests. Soil applications of entomopathogenic nematodes can control plum curculio larvae, e.g., applications of Steinernema riobrave have produced 78-100% suppression. Entomopathogenic fungi also show some promise for control of plum curculio. Entomopathogenic nematodes are virulent to both lesser peachtree borer and peachtree borer. Field applications with S. carpocapsae have resulted in 88-100% peachtree borer control. Additional research is required to incorporate these microbial control tactics into IPM programs.

SYMPOSIUM, Wednesday 14:00 - 16:00

MICROSPORIDIA DIVISION, Microsporidia of Beneficial and Pest insects in Greenhouse, Nursery and Pollination Systems.

Symposium. Wednesday, 14:00. (134)

A unique microsporidium infecting the black vine weevil (Coleoptera: Curculionidae) a pest of landscape, small fruit and nursery plants

Denny J. Bruck1, Leellen Solter2 and 3Michael Baker
1USDA-ARS Horticultural Crops Research Laboratory, 3420 N.W. Orchard Ave., Corvallis, OR 97330, 2Illinois Natural History Survey, 607 E. Peabody Dr., Champaign, IL 61801, and 3Iowa State University, 1184 Molecular Biology Bldg., Ames, IA 50011

We report the first observance of a microsporidian parasite from adult black vine weevil, Otiorhynchus sulcatus, that were originally collected from an ornamental nursery operation in the Willamette Valley of Oregon, USA in the summer of 2003. Based on the ssu-rDNA sequence, this unusual mononucleated microsporidium appears to

be related to Vairimorpha. The microsporidium only infects the fore-, mid- and hindgut of O. sulcatus, causing the gut to become opaque in heavily infected individuals. Subsequent studies to characterize the biology of this organism revealed that it is highly virulent to third instar O. sulcatus; with 80-95% of individuals ingesting as few as 100 spores dying within 12-16 days. Because of this efficacy, applications of 107, 106 and 105 microsporidian spores were made to the surface of 4 inch pots infested with O. sulcatus larvae in the greenhouse. No infection was observed after 7 days; however, infected larvae were recovered from pots drenched with 107 and 106 spores after 14 days exposure. No infection was observed at the 105 spores application rate. Experiments were repeated concentrating on the 107 and 106 spore/pot application rate and an extended recovery period (14 and 21 days). An average of 32% of the larvae recovered from pots receiving 107 spores was infected.

Symposium. Wednesday, 14:25. (135)

Plight of the bumblebee: Pathogen spillover from commercial to wild populations.

Sheila Colla, York University, Toronto, Ontario

Pathogen spread or 'spillover' can occur when heavily infected, domestic hosts interact with closely-related wildlife populations. Commercially-produced bumble bees used in greenhouse pollination often have higher levels of various pathogens than wild bumble bees. These pathogens may spread to wild bees when commercial bees escape from greenhouses and interact with their wild counterparts at nearby flowers. We examined the prevalence of four pathogens in wild bumble bee populations at locations near and distant to commercial greenhouses in southern Ontario, Canada. Bumble bees collected near commercial greenhouses were more frequently infected by those pathogens capable of being transmitted at flowers (Crithidia bombi and Nosema bombi) than bees collected at sites away from greenhouses. We argue that the spillover of pathogens from commercial to wild bees is the most likely cause of this pattern and we discuss the implications of such spillover for bumble bee conservation.

Symposium. Wednesday, 14:45. (136)

The Nosema Riddle: Puzzling fitness effects in the Bumblebee Bombus terrestris

*Otti Oliver and Schmid-Hempel Paul**
Experimental Ecology, Institute of Integrative Biology Zurich (IBZ), ETH Zurich, 8092 Zurich, Switzerland
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Nosema bombi is a microsporidian parasite of bumblebees that is of potentially great ecological and economic importance. However, the understanding of the effects that this parasite has on its host is limited. Recently, N. bombi has been shown to have detrimental fitness effects on one host, the bumblebee Bombus terrestris, under laboratory conditions. This study concludes that under natural conditions bumblebee colonies infected with N. bombi at the start of colony foundation will not reach the size or state sufficient for the production of sexual offspring. To investigate the effects of N. bombi under natural conditions, we conducted a field experiment with B. terrestris spring queens caught after their emergence from hibernation. In the laboratory we assigned these queens to either "infected" (subsequently exposed to N. bombi spores), or "control" treatment groups. On the emergence of workers we placed colonies in the field and measured a number of fitness and life-history traits. We found the size of colonies produced by "infected" queens to be significantly smaller than those of "control" queens. Furthermore, zero of the 14 colonies produced by "infected" queens produced sexual offspring, whereas four of the 14 control colonies reached the male production stage. Our field experiment results are in agreement the laboratory study showing severe effects of N. bombi, and even suggest that such effects are amplified under natural conditions. The evidence suggests that, on entering the colony early, N. bombi has a highly detrimental effect on the fitness of its host. The effect is at such an extent, that it would seem this parasite reduces its opportunities to transmit into the next host generation.

Symposium. Wednesday, 15:05. (137)

The convergent lady beetle and its microsporidium: potential impacts on non-target coccinellids

Taro Saito and Susan Bjornson
Department of Biology, Saint Mary's University, Halifax, NS CANADA

Convergent lady beetles, Hippodamia convergens Guérin-Méneville, are a popular choice for aphid control in North America. In 2004, an unidentified microsporidium was found in H. convergens that were purchased from a commercial insectary. Egg cannibalism (or predation) was examined as a means of horizontal transmission among H. convergens larvae and three field-collected coccinellids of Nova Scotia, including Coccinella septempunctata (seven-spotted lady beetle), C. trifasciata perplexa (three-banded lady beetle), and Harmonia axyridis (multicoloured Asian lady beetle). The microsporidium was transmitted with 100% efficiency in all beetle species examined. Mean spore count data from smear preparations suggested that the infection was as heavy in C. trifasciata perplexa (a native coccinellid) as it was in H. convergens (the natural host) but lighter in C. septempunctata and H. axyridis (introduced species). For all beetle species examined, microsporidia-infected larvae took significantly longer to develop than did uninfected larvae but the microsporidium had no effect on larval mortality. Because the microsporidium in H. convergens is readily transmitted to other coccinellids, field-collected H. convergens should be examined for microsporidia and only uninfected individuals should be used in biological control programs. This is unlikely to happen; however, because H. convergens are inexpensive and easy to collect from their overwintering sites in California.

Symposium. Wednesday, 15:25. (138)

Microsporidia of predaceous mites used for biological pest control

Susan Bjornson
Department of Biology, Saint Mary's University, Halifax, NS CANADA

Microsporidia are common pathogens of natural enemies that are used for pest control in agroecosystems. Microsporidia have been reported from three important species of predaceous mites, including Metaseiulus occidentalis (Nesbitt), Neoseiulus cucumeris (Oudemans), and Phytoseiulus persimilis (Athias-Henriot). These mass-reared mites are made commercially available to growers and home gardeners who depend on them to provide adequate control of thrips and spider mites. Microsporidia are known for causing sub-lethal, debilitating effects that that lower host fitness. In predaceous mites, these effects may be observed as significant reductions in fecundity and adult survival, reduced predation capacity, and male-biased sex ratios. Microsporidia-infected mites may not exhibit any obvious symptoms and because pathogen effects are often subtle, microsporidia may remain unnoticed in mass-rearings until colonies fail to thrive. In predaceous mites, microsporidia are transmitted both horizontally and vertically; however, the mechanisms of transmission have not been determined. Microsporidia may be eliminated from mass-rearings by isolating uninfected individuals (the Pasteur method) and using them to rear uninfected colonies. Microsporidia have also been reduced in colonies when they were reared at elevated temperatures. Healthy colonies were then established from the progeny of heat-treated adults.

Symposium. Wednesday, 15:40. (139)

Dissemination of the biocontrol agent, Vairimorpha necatrix, by the spined soldier bug, Podisus maculiventris.

Rachel Down, Howard Bell, June Matthews & Robert J. Weaver.
Central Science Laboratory, Defra, Sand Hutton, York, UK

The ability of the spined soldier bug, Podisus maculiventris (Say) (Heteroptera: Pentatomidae), to disseminate the infective form of the lepidopteran pathogen, Vairimorpha necatrix (Kramer) (Microspora: Microsporidia) was investigated. Individual female P. maculiventris that had fed on Lacanobia oleracea L. (Lepidoptera: Noctuidae) larvae, infected with V. necatrix, excreted approximately 6 x 108 V. necatrix spores during the subsequent seven days. Excreted spores were fed to L. oleracea larvae, causing 100% mortality, indicating that the spores remained viable after passing through the gut of the predator. Podisus maculiventris that had fed on V. necatrix-infected larvae were allowed to defecate on the foliage of tomato plants, prior to infestation of the plants with L. oleracea or Spodoptera littoralis (Boisduval) (Lepidoptera: Noctuidae) larvae. This proved an effective way of infecting the pest larvae with the pathogen, particularly when five predatory bugs were used per plant. After 20 days, the number of S. littoralis and L. oleracea surviving on the plants was reduced by 75% and 61% respectively. Female P. maculiventris maintained on V. necatrix-infected prey showed reduced egg production and longevity. The potential for P. maculiventris to disseminate insect pathogens will be discussed in the context of improved biological control of lepidopteran pests.

CONTRIBUTED PAPERS, Wednesday 14:00 - 16:00

BACTERIA 3

Contributed Paper. Wednesday, 14:00. (140)

Mutagenic analysis of putative domain II and surface residues in mosquitocidal Bacillus thuringiensis Cry19Aa toxin

Jong Yul Roh1,2, Yeon Ho Je1, and Donald H. Dean,2*
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2Department of Biochemistry, The Ohio State University, Columbus, Ohio 43210, USA.

The mosquitocidal crystal protein, Cry19Aa, from Bacillus thuringiensis subsp. jegathesan, has high toxicity to Anopheles stephensi and Culex pipiens but is less toxic to Aedes aegypti. To study the functional role of domain II and surface residues in mosquito toxicity, 16 alanine substitution mutations were introduced into Cry19Aa. All mutant constructs were expressed as 65 kDa protoxins in Bacillus thuringiensis 4Q7. Subsequent digestion of muteins by trypsin produced fragments of 40 kDa and 25 kDa. With chymotrypsin, however, protoxins were digested to 60 kDa and minor bands. Muteins F325A, Y410A, V420A and T484A, were more sensitive to chymotrypsin than wild-type toxin. The CD spectra of the activated toxins of Cry19Aa and muteins indicated that there was no significant variation in their structure. Mosquito bioassays with Y324A, W357A, Y410A, W416A, D418A and F485A muteins showed toxicity reductions toward A. aegypti and C. pipiens. These mutants also showed reduced competition with wild-type FITC-labeled Cry19Aa for binding to C. pipiens BBMV. These data suggest that the reduction of toxicity was a result of the reduced binding affinity. From these studies we have identified loop residues of domain II that are important in toxicity and receptor binding to Culex larval midgut.

Contributed Paper. Wednesday, 14:15. (141)

Use of by-products rich in carbon and nitrogen as a nutrient source to produce Bacillus thuringiensis based biopesticide.

Fernando H. Valicente1, Alice R. de S. Lopes2 , André H. C. Murão3
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2-Biological Science Student
3-Biological Environment Student

The amount and sources of carbon and nitrogen used to produce Bacillus thuringiensis based biopesticide may influence the quality of the final product. This research used 4 different media using different levels of carbon and nitrogen: medium 1 - 15g/L of maize glucose + 5g/L of soy flour, medium 2 - 30g/L of maize glucose + 10g/L soy flour, medium 3 - 10g/L of maize glucose + 30g/L of soy flour and medium 4- lab commercial Luria Bertani (LB) + salts (FeSO4, ZnSO4, MnSO4, MgSO4). The seed culture was produced using LB medium plus salt, at a stirrer speed of 200rpm, for 18 hours at 30oC. All 4 media were sterilized and inoculated with Bt strain 344 (B. thuringiensis tolworthi). The pH was measured at regular intervals, viable spores were expressed as c.f.u/mL, cell mass produced in g/L-lyophilized, and spore counting per mL of medium. Results showed that the number of spores reached 4.3 x 109 spores/mL in medium 3, where the amount of protein is high. The same pattern was observed with the cell mass production where media 3 produced more than 3.0g/L (non-cumulative). Media 3 and 4 killed 100% of 2 day old Spodoptera frugiperda larvae.

Contributed Paper. Wednesday, 14:30. (142)

Phagocytic activity and encapsulation rate of Galleria mellonella larvae hemolymph during bacterial infection Bacillus thuringiensis

Dubovskiy I.M., Krukova N.A., Grizanova E.V., Naumkina E., Glupov V.V.
Institute of systematics and ecology of animals SB RAS, Russia, Novosibirsk

The entomopathogenic microorganisms play essential role in the dynamics of insects population in both natural ecosystems, and agrocoenosis. The bacteria Bacillus thuringiensis (Bt) is a pathogen of many species of insects and is actively used in biocontrol. After peroral inoculation of G.mellonella by Bt in low concentration (LC5) we registered 1.5 fold increase of phagocytic activity of infected larvae to compare with control larvae on the second and third days after inoculation. The encapsulation rate of nylon implants in haemolymph did not change. When the concentration of bacterial cells was corresponded to LC15 we have revealed the double increase of phagocytic activity and encapsulation rate in haemolymph of infected insects on the second and third days of experiment. It is necessary to note that increase of phagocytic activity on the second and third days after inoculation with LC15 dose was more expressing in contrast with using lower dose of bacteria.

Summarizing, we have shown that larvae infected with sublethal doses of Bt had increasing phagocytic and encapsulation activities. Probably the destructions of the midgut epithelium cells and following discharge in lymph factors activating immune reactions (lipophorin, eicosanoids, calcium) during the bacteriosis may lead to increase of cellular immunity.

Contributed Paper. Wednesday, 14:45. (143)

AFM imaging of Bacillus thuringiensis Cry1 toxins interacting with insect midgut apical membranes

Éric Laflamme1,2, Antonella Badia1,3, Michel Lafleur1,3, Jean-Louis Schwartz1,4 and Raynald Laprade1,2
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Atomic force microscopy was used to image Bacillus thuringiensis (Bt) toxins interacting with Manduca sexta midgut brush border membranes, their natural target in this lepidopteran pest, as well

as with DPPC-DOPC solid-supported lipid bilayers. In lipid bilayers, Cry1Aa formed structures 30- by 60-nm wide and 3- to 7-nm high, mostly at the interface of domains formed by the two different lipids or at the edge of DOPC domains. Brush border membrane vesicles, in the absence of toxin, formed flat membrane fragments of up to 25 μm2 and 4.2-nm high, with irregular embedded structures. After incubation with Cry1Aa, Cry1Ac and Cry1C, which are active against M. sexta, new structures, 35-nm wide and 5.1- to 6.7-nm high, were observed in some membrane fragments, sometimes only in particular regions. Their density, which reached a plateau within 4 h, depended on the toxin and its concentration. The structures formed by Cry1Ac were often grouped into dense, two-dimensional arrangements. No such specific interactions were observed with Cry1Ba, which is inactive against M. sexta. This study provides the first visual evidence of specific interactions of Bt toxins with insect midgut brush border membrane fragments at the nanometric scale. The observed structures may represent the protein complexes forming functional Bt pores in target membranes.

Contributed Paper. Wednesday, 15:00. (144)

Quantitative Cry toxin binding analyses using time resolved fluorescence

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Binding of Cry toxins from Bacillus thuringiensis to specific receptors on the insect midgut epithelium is required for toxicity. Analyses of Cry toxin binding to midgut brush border membrane vesicles (BBMV) have facilitated the development of toxin binding site models and the characterization of resistance mechanisms. Radioiodinated Cry toxins have been widely used in quantitative binding competition assays. However, some Cry toxins are adversely affected by radioiodination, and there is a need for alternative labeling and binding procedures to facilitate binding studies using these toxins. We present methodology to label Cry toxins with lanthanide derivatives and perform quantitative binding assays using time resolved fluorescence (TRF). Bioassays with neonate Heliothis virescens larvae demonstrate that lanthanide labeling does not affect toxin activity. Our data demonstrate the utility of this fluorescent approach to perform quantitative toxin binding assays with toxins affected by radioiodination.

Contributed Paper. Wednesday, 15:15. (146)

A rapid and highly sensitive assay for evaluating Bacillus thuringiensis strains for their insecticidal activity toward target insect pests.

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Bacillus thuringiensis (Bt) pore-forming toxins induce rapid and massive release of membrane-bound aminopeptidase-N (APN) into the gut fluid in the gypsy moth, Lymantria dispar. This discovery led to the development of a simple, rapid and highly sensitive assay for screening Bt toxins. The amount of soluble APN released was found to be dose-and time-dependent and a reliable measure of the potency of various Bt samples. Using this assay, we have found all insecticidal proteins tested to date induce APN release, whereas heat-denatured or inactive Bt samples do not. Furthermore, this highly sensitive assay can be used to evaluate the activity of crude Bt culture samples grown on nutrient agar plates. The major advantages of this technique over the traditional bioassay are that it is fast (results can be obtained in hours instead of days) and highly sensitive. The sensitivity is such that it can detect the effects of sublethal doses of Bt samples, thereby overcoming a problem with insects that are not very sensitive to purified Cry proteins without the synergistic effect of other pathogenic factors such as spores.

Contributed Paper. Wednesday, 15:30. (147)

Structural changes of the Cry1Ac oligomeric pre-pore from Bacillus thuringiensis induced by N-Acetylgalactosamine facilitates toxin membrane insertion†

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The primary action of Cry toxins produced by Bacillus thuringiensis is to lyse midgut epithelial cells in their target insect by forming lytic pores. The toxin-receptor interaction is a complex process,involving multiple interactions with different receptor and carbohydrate molecules. It has been proponed that Cry1A toxins sequentially interact with a cadherin receptor, leading to the formation of a pre-pore oligomer structure, and that the oligomeric structure binds to glycosylphosphatidyl-inositol-anchored aminopeptidase-N (APN) receptor. The Cry1Ac toxin specifically recognizes the N-acetylgalactosamine (GalNAc) carbohydrate present in the APN receptor from Manduca sexta larvae. In this work, we show that the Cry1Ac pre-pore oligomer has a higher binding affinity with APN than the monomeric toxin.

The effects of GalNAc binding on the toxin structure were studied in the monomeric Cry1Ac, in the soluble pre-pore oligomeric structure, and in its membrane inserted state by recording the fluorescente status of the tryptophan (W) residues. Our results indicate that the W residues of Cry1Ac have a different exposure to the solvent when compared with that of the closely related Cry1Ab toxin. GalNAc binding specifically affects the exposure of W545 in the pre-pore oligomer in contrast to the monomer where GalNAc binding did not affect the fluorescence of the toxin. These results indicate a subtle conformational change in the GalNAc binding pocket in the pre-pore oligomer that could explain the increased binding affinity of the Cry1Ac pre-pore to APN.

CONTRIBUTED PAPERS, Wednesday 14:00 - 16:00

VIRUSES 4, Virus Production, Infection and Biotechnology

Contributed Paper. Wednesday, 14:00. (148)

Translation of complex baculovirus mRNAs: an unanswered question?

Ian Smith
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Baculovirus genomes encode ~90-180 polypeptides, and recent microarray data suggest that all of their genes may be expressed during the course of infection. There appears to be a tendency for transcripts at particular loci to become progressively longer as infection proceeds, so that late mRNAs may encompass more than one open reading frame (ORF). For a given gene, these longer 'polycistronic' transcripts may, eventually, greatly outnumber the single-ORF monocistronic population. This phenomenon affects how we should interpret transcriptional array data: gene expression involves translation as well as transcription, and it may be erroneous to assume that the transcriptional array profile for a particular ORF reflects synthesis of the corresponding polypeptide. As a simple example, an mRNA having the structure 5'-ORFA-3' should yield polypeptide A. At later times, however, this mRNA may be superseded by longer transcripts such as 5'-ORFB-ORFA-3' and 5'-ORFC-ORFB-ORFA-3'. Can polypeptide A still be synthesized from such messengers, or is only the ORF closest to the 5' terminus translatable? I will argue that the available data suggest that the

answers to this bipartite question are 'no' and 'yes', respectively. If these answers are correct, the value of baculovirus transcriptional array data as indicators of gene expression becomes doubtful.

Contributed Paper. Wednesday, 14:15. (149)

Identification of retroviruses in insect cells used for baculovirus expression

Tila Menzel and George F. Rohrmann

Insect retroviruses have a novel evolutionary relationship with baculoviruses. Evidence indicates that they originated when a transposable element from an insect cell integrated into and obtained an envelope gene from a baculovirus via a recombination event. In addition, a lepidopteran retrovirus that was found integrated into a baculovirus genome had a baculovirus late promoter element in its LTR such that it it could be expressed at high levels late in the baculovirus infection cycle. Both these observations attest to a potentially complex, but largely uncharacterized relationship between baculoviruses, insect retroviruses, and their host cells. In order to develop an understanding of the number and diversity of lepidopteran retroviruses, PCR using primers complementary to conserved regions of the retrovirus reverse transcriptase gene in conjunction with DNA cloning and sequencing was used to isolate sequences from Spodoptera frugiperda (Sf-9) and Trichoplusia ni (Hi-5) cell lines. Phylogenetic analysis of over 20 sequences from each cell line indicates the presence of a diverse population of endogenous retroviruses in both types of cells.

Contributed Paper. Wednesday, 14:30. (150)

Establishing a Tissue Culture System for the Mosquito Iridescent Virus (RMIV) from Ochlerotatus taeniorhynchus

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Mosquito iridescent viruses (MIV) are members of the genus Chloriridovirus which currently contains only the type IIV3 from Ochlerotatus taeniorhynchus (designated as RMIV). With the recently published DNA sequence and analysis of RMIV, the complete genomes have been determined for viruses representing all IV genera. It is currently recognized that MIVs are distinct from all known iridescent viruses and that the diversity among invertebrate iridescent viruses may be greater than previously recognized. The ability of MIV to infect a mosquito cell line was first demonstrated in a series of studies from 1974-1976 when Webb and colleagues infected 2 cell lines of Aedes aegypti. Now that the genome of RMIV has been sequenced, there is a need for a tissue culture system for RMIV. Investigations have been initiated to establish RMIV in mosquito cell lines of Aedes aegypti (Aag2), Ae. albopictus (C6/36) and Anopheles gambiae (4a3A) to determine whether these lines are permissive and the optimal conditions for growth. The availability of a vigorous cell culture system is crucial for conducting functional genomic studies on RMIV.

Contributed Paper. Wednesday, 14:45. (151)

Production of LdNPV in the Wave® cell culture bioreactor: Comparison to production in a stirred tank bioreactor

James M. Slavicek
USDA Forest Service, Northern Research Station, Delaware, Ohio

A bioreactor with a novel design is increasing being used to produce foreign proteins in insect cells. This bioreactor, the Wave® bioreactor, has several advantages compared to a stirred tank bioreactor. Methods to produce the Lymantria dispar nucleopolyhedrovirus (LdNPV) in the Wave® bioreactor were developed at 5 and 10 liter scales, and polyhedra production levels of approximately 4 x 1010 polyhedra per liter were obtained in both the 5 and 10 liter bags. A comparison of polyhedra production in the Wave® vs. stirred tank bioreactors found that the Wave® system produced about 2 fold more polyhedra in all studies compared to the stirred tank bioreactor. During the course of our recent studies in both types of bioreactors, a steady decline in polyhedra production was found that stabilized at a level of about 10-fold less than earlier production levels. Studies on this decline indicated that the probable reason is that the maximum achievable cell densities in the bioreactors declined from 8 x 106 cells/ml to only 2 x 106 cells/ml. During these studies the morphology of the Ld652Y mixed cell line changed from being primarily fibroblast-like to spherical. Efforts to address the problem of a changing cell line will be discussed.

Contributed Paper. Wednesday, 15:00. (152)

Insecticidal activity of the baculovirus expressed, basement membrane-degrading protease, ScathL

Hailin Tang1, Huarong Li1, S. Sivakumar1, Robert L. Harrison2, John A. Gatehouse3, Bryony C. Bonning1
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ScathL is a cysteine protease that functions in the remodeling of basement membrane during insect metamorphosis. Baculovirus expression of ScathL results in rapid mortality of the tobacco budworm, Heliothis virescens. Here, we report on the insecticidal action of ScathL when delivered either by a recombinant baculovirus or by injection. Melanization followed by the rapid death of larvae infected by the recombinant baculovirus AcMLF9.ScathL was consistently associated with high levels of ScathL in the hemolymph of fifth-instar larvae. Fragmented fat body tissue, a ruptured gut, and melanized tracheae were observed in AcMLF9.ScathL-infected larvae. ScathL is remarkably specific and appears to result only in disruption of the basement membrane when delivered by a baculovirus, followed by loss of integrity of the underlying tissues. Following injection of purified ScathL, larvae exhibited similar internal tissue damage with an LD50 of 10.99 µg/larva for fifth-instar H. virescens. These results demonstrate one possible mechanism by which baculovirus-expressed ScathL may kill host larvae.

Contributed Paper. Wednesday, 15:15. (153)

Impact of a basement membrane-degrading protease on dissemination and secondary infection of Autographa californica multiple nucleopolyhedrovirus in Heliothis virescens (Fabricus)

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ScathL is a cathepsin L-like cysteine protease derived from the flesh fly, Sarcophaga peregrina that digests components of the basement membrane during insect metamorphosis. A recombinant baculovirus that expresses ScathL (AcMLF9.ScathL) kills larvae of the tobacco budworm, Heliothis virescens, significantly faster than the wild-type virus and triggers melanization and tissue fragmentation in infected larvae shortly before death. Since basement membranes are a potential barrier to the spread of baculovirus secondary infection to other tissues in the host, we tested the hypothesis that the rapid death of insects infected with AcMLF9.ScathL was caused by accelerated secondary infection resulting from the degradation of host basement membranes by ScathL. Viruses expressing catalytically active or inactive ScathL were used to examine the effects of ScathL activity on budded virus release into the hemocoel during infection, the production of polyhedra in infected larvae, and the rate of infection of the gut, trachea, hemocytes, fat body, and malpighian tubules. We

conclude that the enhanced insecticidal efficacy of the recombinant baculovirus that expresses ScathL does not result from altered tissue tropism or accelerated systemic infection. Implications for the role of the basement membrane as a barrier to baculovirus dissemination within the host insect will be discussed.

Contributed Paper. Wednesday, 15:30. (154)

Infection of two lepidopteran cell lines with Amsacta moorei entomopoxvirus and induction of apoptosis

Srini Perera1, 2, Philip Wong3, Kathleen Rossi2, Peter Krell2 and Basil Arif1

1Great Lakes Forestry Centre, Sault Ste. Marie, ON, Canada. 2 Department of Microbiology, University of Guelph, ON, Canada. 3Queens University, ON, Canada.

The Amsacta moorei entomopoxvirus (AmEVAMEV) has been previously shown to establish a successful infection in the gypsy moth (Lymantria dispar) cell line IPLB-Ld652. We have compared the infectivity of AmEVAMEV in Ld652, and in FPMI-Cf70-B2 which is a clonal cell line derived from the spruce bud worm, Choristoneura fumiferana. Both cell lines supported high levels of budded virus production as determined by TCID50 assays. However, occlusion body production was lower in Cf70-B2 cells. Subsequent passaging (up to four passages) showed a slight decline in budded virus titers in both cell lines. Despite the high levels of budded virus production, Cf70-B2 cells underwent apoptosis as a result of AmEVAMEV infection. At late stages of infection, cell membrane blebbing and formation of apoptotic bodies were visualized by light microscopy. Increased levels of caspase-3-like activity were also observed in Cf70-B2 cells infected with AmEVAMEV as compared to uninfected cells. However, there was no indication of cellular DNA fragmentation in infected Cf70-B2 cells. Interestingly, Ld652 cells, which are fully permissive to infection also showed symptoms of apoptosis such as DNA fragmentation and induction of caspase-3-like activity. The expression of anti-apoptotic genes AMVp33 and AMViap in the two cell lines is currently being investigated.

Contributed Paper. Wednesday, 15:45. (155)

Isolation and characterisation of the Serratia entomophila anti-feeding prophage - a unique toxin delivery system?

Mark RH. Hurst1, Sam S. Beard1, Trevor A. Jackson1 & Sandra M. Jones1

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The Serratia entomophila anti-feeding prophage (Afp), forms a virus-like structure that has causes cessation of feeding in the New Zealand grass grub, Costelytra zealandica. Through the trans based expression of AnfA1, a RfaH –like transcriptional anti-terminator, the Afp was induced. The expressed Afp was purified, and visualised by electron microscopy. The Afp resembled a phage tail-like bacteriocin, exhibiting two distinct morphologies, an extended and contracted forms. When fed to C. zealandica larvae, the purified Afp caused cessation of feeding and a change to an amber colouration within 48 hours from ingestion, with increased dose rates causing larval mortality. Electron micrographs of various AFP forms will be presented in conjunction with a proposed model for toxin delivery.

Wednesday 16:30 - 18:30, Pavillon Vachon, 2nd Floor

POSTERS - II

FUNGI

Poster / Fungi. F-01

Beauveria: The emergence of a new classification

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The harmonization of traditional and molecular approaches to the taxonomy of so large and universally important a genus of fungal pathogens as Beauveria is now becoming a reality. Critical re-examination of the traditionally accepted characters of several hundred isolates of Beauveria from the ARSEF culture collection for which multigenic sequence data have been generated and analyzed phylogenetically have made it possible to recognize the despite the general morphological similarities among the genetically divergent fungi in this genus, there are still some surprising correlations of morphological characters, and even some new and previously undescribed developmental behaviors have been documented. Rehner and Buckley (2005. Mycologia 97: 84-98) found that isolates identified as B. bassiana distributed themselves genotypically into two wholly separate clades despite their similar morphologies: The larger and still more genotypically diversified clade comprises B. bassiana in the strict sense (since this clade includes the fungus being designated as the neotype for B. bassiana) is morphologically distinguished from the second clade which will be described as B. pseudobassiana. Another new species distinguished by its genotype comprises a cluster of Asian isolates known to have a Cordyceps teleomorph, and will be described as B. sungii.

Poster / Fungi. F-02

Endophytic and entomopathogenic characteristics of a fungus in the genus Colletotrichum

Marcelino, J., Gouli, S., Gouli, V., Giordano, R., Parker, B., Skinner, M.

We report the presence of an ubiquitous strain of the fungus Colletotrichum sp. within areas of a natural occurring epizootic in populations of the Northeastern USA Fiorinia externa, elongate hemlock scale. The host range of this Colletotrichum strain comprises both insects and plants, although different life styles occur in the different hosts. Endophytic growth was observed in 26 species of plants comprising 18 families (52 % of the sampling), whereas in F. externa biotrophic and necotrophic growth was detected. Colletotrichum is a widely known phytopathogenic genus and reports of entomopathogenic activity are extremely rare. In order to understand the biological processes involved in the host-pathogen interactions we quantified the spatio-temporal progression of disease in the different hosts. Hemibiotrophic and subsequent biotrophic growth was induced in laboratory condition in strawberry plant leaves, as well as, in an axenic population line of F. externa. We observed that under suitable laboratory conditions this Colletotrichum sp. strain is capable of inducing fast-developing mortality rates in both hosts, suggesting a high plasticity in the infection strategies of this strain which can be related to the adaptation of the fungus to a novel insect host.

Poster / Fungi. F-03

Surface properties of Beauveria bassiana single cell types

Diane Holder, Brett Kirkland, and Nemat O. Keyhani

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Surface characteristics of aerial conidia, in vitro blastospores, and submerged conidia, three types of single cell propagules produced by B. bassiana, were analyzed by Atomic Force Microscopy (AFM), zeta potential measurements, contact angle determinations using polar and apolar test liquids, and microbial adhesion to hydrocarbons (MATH). These data revealed marked differences in surface topology, net charge, and hydrophobicity between the cells. AFM

revealed the presence of a rodlet layer in aerial conidia that was absent from in vitro blastospores and submerged conidia. Overall, the surfaces of aerial conidia were hydrophobic and displayed large net surface charge variation at pH values ranging from 3 to 9. Surfaces of in vitro blastospores hydrophilic and displayed the smallest variation in surface charge over the same pH range, whereas submerged conidia displayed cell surface characteristics at the border between hydrophobic and hydrophilic, as well as intermediate net surface charge variation over pH values ranging from 3 to 9. Insect pathology assays using tobacco budworm (Heliothis virescens) indicated some variation in virulence between the B. bassiana cell types using both topical application and hemoceol injection of the fungal cells.

Poster / Fungi. F-04

Genetic and phenotypic variation in Metarhizium anisopliae isolated from golf courses in Québec

Parivash Shoukouhi1, Louis Simard2, Guy Bélair2 and John Bissett1

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Metarhizium anisopliae was the predominant fungal species (140/211 isolates) isolated from baited Galleria mellonella and Tenebrio molitor in soil samples from golf courses in Quebec. Six genotypes evolved from two separate and distinct lineages were determined from sequences of the internal transcribed spacer regions of rDNA (ITS1-2), and a 0.9 kb fragment of the translation elongation factor gene (eEF1a1). No clear geographic pattern for distribution of genotypes was observed, with all six genotypes well represented, for example, in the Montréal-Montérégie regions with the largest concentration of golf courses. However, the Abitibi-Témiscamingue region was represented by a single genotype, and a second genotype was clearly predominant in the Bas St. Laurent-Gaspésie regions. One genotype was isolated only from T. molitor ; all other genotypes were isolated from both G. mellonella and T. molitor . Metabolic profiling using Biolog FF MicroPlatesTM clearly differentiated the two lineages, as well as three of the six genotypes. The remaining three genotypes, all from the same lineage, had similar metabolic profiles, despite evidence of differences in their distribution patterns. We conclude that environmental and geographic factors, as well as host affinities, all may have contributed to evolution of divergent strains within the M. anisopliae aggregate species in Québec.

Poster / Fungi. F-05

Morphological and molecular characterization of some new entomopathogenic fungi originating from soils in Central Brazil, and their activity against Triatoma infestans

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Fungi identified as Evlachovaea sp have been recently reported from Brazil and one isolate found on a dead Triatoma sordida showed promising activity against Triatoma infestans, which transmits Chagas disease. In this study the isolates IP67, IP126, IP141, IP142, IP148, IP154, closely resembling the original isolate, were obtained from soils collected in Central Brazil with T. infestans as bait insects. All isolates were morphologically identified as Evlachovaea sp in a genus whose only described species is E. kintrischica. The rDNA ITS sequence of IP126 and IP148 was most similar to Isaria fumosorosea (ARSEF1576). The other isolates were identical in ITS sequence and were closest to a Beauveria bassiana (Genbank Z54111). Interestingly, the ITS sequence of E. kintrischica ARSEF7218, was identical to Isaria amoenorosea CBS738.73, which calls into doubt the taxonomic validity of the genus Evlachovaea. All of these new isolates were shown to be active against T. infestans at 25°C and RH >98%. However, only IP141 induced significant mortality at RH 75%. Lethal time 90% varied from 8d (IP141) to 22.8d (IP67) at RH >98%. Our results underline the need for more extensive studies on the taxonomy of these fungi and their potential for control of triatomines.

Poster / Fungi. F-06

Entomopathogenic fungi infecting the Asian citrus psyllid, Diaphorina citri (Homoptera: Psyllidae), in Florida

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The Asian citrus psyllid, Diaphorina citri is an invasive pest that vectors citrus greening disease. In 2005-2006 mycosed psyllids displaying two phenotypes were collected in central Florida. The major pathogen, identified by morphological and genetic analyses, was a novel isolate related to Hirsutella citriformis. In vitro cultures of the fungus were slow-growing and produced synnemata similar to those found on mycosed D. citri. In laboratory bioassays, high levels of mortality were observed in D. citri that were exposed to the conidia-bearing synnemata produced in vivo and in vitro. Infected psyllids had an abundance of septate hyphal bodies in their hemolymph and exhibited behavioral symptoms of disease. Isolate-specific PCR primers were designed to detect the pathogen in seasonal samples of psyllid populations. The second pathogen, identified as an isolate of Paecilomyces fumosoroseus (Pfr), was differentiated from the Florida isolate Pfr97 by amplified fragment length polymorphism (AFLP) analysis and by in vitro growth characteristics. Healthy adult D. citri from a laboratory colony died within 72 hr after exposure to field-collected cadavers and in vitro cultures of Pfr ACP. A series of arthropods were susceptible to infection with Pfr AsCP, indicating that this pathogen has a broad host range.

Poster / Fungi. F-07

Surveys of indigenous entomopathogenic fungi and nematodos of Chile and studies on their pathogenicity towards pests of economic importance

Loreto Merino , Steve Edgington , Dave Moore2 y Andrés France1.

The Insect Pathology Program at INIA Quilamapu (Chile) is working in collaboration with CABI (UK) on a Darwin Initiative (DEFRA-UK) to conserve and use entomopathogenic microorganisms in Chile. The aim is to collect entomopathogenic fungi and nematodes from some of the major ecological habitats in Chile. Six survey transects have been chosen: 1. Latitude 20o, with sections of Altiplano and on the periphery of the Atacama Desert; 2. Latitude 30o, desert with remnants of ancient tropical forests; 3. Latitude 33o, an area of Mediterranean vegetation; 4. Latitude 37o, a transitional zone from dryland into wetland; 5. Latitude 46o, heavy rainfall, relatively cold, with humid forests and areas of pampas, 6. Latitude 52o, Tierra del Fuego, with near Antarctic conditions and flora and fauna adapted to low temperaturas.

The first two transects have been surveyed, revealing 157 isolates of entomopathogenic fungi, predominately Metarhizium and Beauveria spp. and 14 isolates of nematode, Heterorhabditis and Steinernema spp. The isolates will be placed into the Genetic Resource Collection at INIA, significantly enhancing the bank of indigenous germplasm already present. It is likely that indigenous isolates will

show stronger adaptations to conditions in Chile compared to exotic isolates and could be important pest control options.

Poster / Fungi. F-08

Occurrence of invertebrate-pathogenic fungi in a Cerrado ecosystem in Central Brazil

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The Cerrado is a major Brazilian biodiversity that is being seriously threatened by human activities. Little information exists about entomopathogenic fungi and other beneficial microorganism in this ecoregion. We report about the natural occurrence of invertebrate-pathogenic fungi in a typical but protected tropical gallery forest area in the Cerrado near Goiânia in Central Brazil. A total of 73 fungal isolates were obtained from water, sediment and soil samples collected during the dry season in 2006. Fungi were baited from these substrates using Aedes aegypti, Culex quinquefasciatus, Rhodnius neglectus, Boophilus microplus and a snail, Biomphalaria glabrata. Resulting isolates were identified morphologically as isolates of Aspergillus, Beauveria, Cladosporium, Evlachovaea, Fusarium, Gliocladium, Isaria, Lecanicillium, Metarhizium, Paecilomyces, Pochonia, Sporothrix and Trichoderma. Laboratory bioassays proved several Fusarium, Gliocladium, Lecanicillium psalliotae, Paecilomyces lilacinus and Pochonia chlamydosporia isolates were active against triatomines. During the rainy season in October, this forest site yielded a major epizootic of a Batkoa species on small nematoceran flies and other insects killed by Aschersonia, Beauveria, Fusarium, Hirsutella, Lecanicillium, Cordyceps, Hypocrella, Torrubiella and Pandora. These results confirm an elevated biodiversity of invertebrate-pathogenic fungi in this preserve and emphasize the need to intensify studies about these fungi and their potential for pest control.

Poster / Fungi. F-09

Detection of chalkbrood of honeybees (Apis mellifera) caused by the fungus Ascosphaera apis

Sung Hee Nam, Ji Young Choi, Myeong Lyeol Lee, In Pyo Hong, Kyu byoung Sung and Chung In Mo

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The pathogenic fungus Ascosphaera apis is the causative agent of the chalkbrood disease in honey bee, Apis melifera. This disease occurs through the world and is found in many beekeeping area of Korea. Infected larvae of honey bee were obtained from 8 bee farms in Korea. They are 548 samples with varying numbers of black and white mummies of honey bee. Among them, 306 black mummies were examined to identify species. It only infects larvae that are three to four days old. The environment of disease occurrence is humid and high temperature in the hives. Dead brood and pathogens of A. apis were founded in the combs and on the ground around hives. Up to 80% of a brood can be killed by chalkbrood disease, resulting in infected hives dying out. But there are no chemical treatments for this disease.

In this study, PCR methods for identification and rapid detection of the pathogene have now been extensively developed. Honeybee larva, hive, soil, debris, pollen and their breeding environment were tested by polymerase chain reaction(PCR). The technique can be directly used to detect presence or absence of A. apis spores in honey bee brood samples and contaminated environment.

Poster / Fungi. F-10

Annotated checklist of arthropod-pathogenic fungi from Brazil and Argentina

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We present an updated list of arthropod-pathogenic fungi occurring in Argentina (from 12 provinces) and Brazil (from 15 states) based on published literature and our personal observations. The list includes at least 63 species and three varieties of fungi from 33 genera of Entomophthorales (Conidiobolus, Batkoa, Entomophaga, Entomophthora, Furia, Pandora, Zoophthora, Neozygites), Trichomycetes (Smittium, Harpella, Genistellospora), Ascomycetes (conidial states in Aspergillus, Aphanocladium, Aschersonia, Beauveria, Cladosporium, Clonostachys, Evlachovaea, Fusarium, Gibellula, Hirsutella, Isaria, Lecanicillium, Metarhizium, Nomuraea, Paecilomyces, Tetracrium, and sexual states in Cordyceps, Myriangium, and Ascosphaera), Chytridiomycetes (Coelomomyces, Coelomycidium), and Oomycetes (Leptolegnia). Whenever possible, the most relevant features, photographs, host species and bibliographic sources are provided. Pathogenicity remains uncertain for some species of Cladosporium and Trichomycetes (whose species may have commensal, mutualistic or pathogenic associations with their hosts). This list includes at least three undescribed new species. Most of these fungi affect insects but a few are mite or spider pathogens. The arthropod hosts are agricultural pests or insect vectors of significant diseases. Cultures are deposited in collections in Argentina, Brazil and USA. Some records included here need confirmation or corrections, and additional information will be welcomed.

Poster / Fungi. F-11

Difference in aphid and fly host driven divergence of Entomophthora species

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Several species of the genus Entomophthora infect higher dipterans (Cyclorrhapha): E. ferdinandi, E. grandis, E. muscae, E. scathophagae, E. schizophorae and E. syrphi. In comparison, only two aphid pathogenic Entomophthora species, E. chromaphidis and E. planchoniana have been described. Molecular analyses have recently confirmed the species status of most of the fly pathogenic Entomophthora, while it has been questioned whether the two aphid pathogenic Entomophthora species are two distinct species, since they cannot be distinguished molecularly. In addition, molecular analyses have revealed high intra-specific variation within E. muscae showing that each host species harbor its own fungus genotype. In the current study we sequenced several DNA regions of Entomophthora origination from different fly and aphid host taxa. The results documented a huge genetic divergence of the fly pathogenic Entomophthora in comparison to the aphid pathogenic Entomophthora, where only minor differences in the sequences where detected. The evolutionary time of divergence of the fly and the aphis host taxa included in this study cannot account for this difference. The host driven divergence of Entomophthora is therefore much higher in flies compared to aphids. Different hypotheses for this difference will be discussed.

Poster / Fungi. F-12

Can Metarhizium anisopliae, really colonize the plant rhizoplane?

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The entomopathogenic fungus Metarhizium anisopliae Strain F52 is being developed for biocontrol of the sugarbeet root maggot. Incorporating this fungus in a seed coat for subsequent root colonization could be an ideal approach in using this fungus. The ability of GFP-transformed Metarhizium to colonize sugarbeet roots was observed with seeds coated with fungal spores, only when an axenic agar culture method was employed. Root colonization by F52 was not observed on sugarbeet seedlings grown in sterile vermiculite wetted with dilute Hoagland's medium. No colonization was observed on the roots of table beets, chard, spinach, cabbage, corn or beans in the vermiculite-Hoagland's system, although GFP-expressing hyphal growth was observed on bean and corn seeds. Germination of conidia, a prerequisite for root colonization, was plant-stage dependent in sugarbeet root exudate. Spores did not germinate when root exudate from cotyledons was used as the medium, but a high germination rate occurred when exudate was from plants with 2-4 leaves. A high germination rate also occurred in exudates from oats, rye, beans, cabbage, and chard. While substantial hyphal growth occurred in oat, rye, bean and chard root exudates, it did not occur in cabbage or sugarbeet root exudates.

Poster / Fungi. F-13

Inhibition of phagocytic activity and nodulation in Galleria mellonella by the entomopathogenic fungus, Nomuraea rileyi

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Changes in phagocytic activity and nodulation in the greater wax moth, Galleria mellonella, were examined after treatment with the cultured fluid of the entomopathogenic fungus, Nomuraea rileyi SH1. When incubating the isolated hemocytes with conidia of N. rileyi in vitro, phagocytic rates elevated at 4 h after incubation but decreased thereafter. In contrast, the phagocytic rate of the isolated hemocytes decreased 80% after pre-incubation with 1/200 and 1/100 dilutions of the fungal cultured fluid for 24 h. Phagocytic activity was inhibited by the cultured fluid in a dose-dependent manner. Larvae of G. mellonella showed a peak of nodulation at 4 h after injection with conidia. The percentage of nodules in hemolymph was not decreased by pre-injection with the cultured fluid, while the percentage of nodules containing conidia decreased, depending on the dose injected. However, phagocytosis and nodulation in G. mellonella remained normal after treatment of the cultured fluid with proteinase K, indicating that the cultured fluid contains toxic proteinaceous substance(s). In summary, N. rileyi could release toxic proteins to impair the cellular immune responses in G. mellonella larvae and is therefore pathogenic to lepidopterous insects.

Poster / Fungi. F-14

Pathogenicity and its mode of action of Verticillium lecanii (Lecanicillium spp.) hybrid strains against different sedentary stages of Heterodera glycines

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The current study was conducted in order to investigate pathogenicity of hybrid strains of V. lecanii against different sedentary stages of Heterodera glycines and its mode of action. Three different stages (pale yellow female; light brown cyst; and dark brown cyst) of H. glycines were treated with V. lecanii and incubated for 3 weeks on water agar. After 3 weeks incubation, eggs were collected and investigated the following subjects: 1) Infection frequencies of eggs; 2) Number of egg-laying; and 3) Number of mature and healthy eggs. Subsequently, the fecundity of H. glycines treated with some of the hybrid strains was investigated in greenhouse pot test. As a result of these experiments, some hybrid strains infected eggs at high frequency and significantly reduced the number of egg-laying and mature eggs in vitro tests. Furthermore, it was observed that several strains reduced the number of eggs/cysts in greenhouse test. These results suggested that V. lecanii could infect the females of H. glycines and reduced their fecundity. In conclusion, this study indicated that V. lecanii was more effective for females than cysts, and V. lecanii could act against H. glycines by the multiple ways.

Poster / Fungi. F-15

Pathogenicity of Verticillium lecanii (Lecanicillium spp.) hybrid strains to different developmental stages of greenhouse whitefly, Trialeurodes vaporariorum

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The fungus Verticillium lecanii was investigated as a possible biological control agent for greenhouse whitefly. To obtain more useful strain, Aiuchi et al. (2004) conducted to protoplast fusion, among three strains (Vertalec®, Mycotal® and B-2). Pre-selected 11 hybrid strains and 3 parental strains were tested for their pathogenicity to various developmental stages of Trialeurodes vaporariorum (Aiuchi et al., 2007). Mortality of larvae recorded on 3 days post inoculation (dpi). The most virulent strains reveal 30-fold-decreases median lethal concentrations (LC50) compared with Mycotal at 5dpi. LT50 of high virulent strains were half time (4.22, 4.48 days) of Mycotal. Higher mortality caused by hybrid strains (2aF4, 2aF43, 2aF30, 2aF31) was over 80%. There were no effects on hatchability of T. vaporariorum after fungal inoculation, but mortality of hatching larvae were ca. 95% (Vertalec, B-2, 2aF1, 2aF4 2aF13, 2aF33, 2aF36 and 2aF43 at 1.0×107 conidia/ml). Among these strains, 2aF1, 2aF43, 2aF4, 2aF26 showed 10-fold-decreases in LC50 compared with Mycotal. These larvae became cadaver immediately after hatching. These results suggested that high pathogenicity strain might affect to larvae in the eggs. Finally 3 hybrid strains, 2aF43, 2aF1, and 2aF30 revealed high pathogenicity to each developmental stage.

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Poster / Fungi. F-16

Susceptibility of immature stages of the greenhouse whitefly parasitoid, Encarsia formosa, to the entomopathogenic fungus Verticillium lecanii

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The parasitoid Encarsia formosa and entomophagus fungus Verticillium lecanii show a high potential for controlling greenhouse whitefly, Trialeorudes vaporariorum. However, probably there are antagonistic effects in integrated use of them. In this study, different aspects of pathogencity of V. lecanii (DAOM 198499) were

evaluated on parasitoid, E. formosa, under laboratory conditions. Larva of E. formosa inoculated with the doses of 104 (sub-lethal), 1/8*105 (LC50) and 108 (lethal) conidia/ml of fungal suspensions (estimated for greenhouse whitefly) and sterile distilled water as control. Analysis of data indicated that mean mortality was 14/14%, 28/38% and 88/87% in 104, 1/8*105 and 108 conidia/ml respectively. Effect of V. lecanii was also evaluated on pupa stage of E. formosa with the same concentration as above. Mean mortality of 16/25% in 104, 17/88% in 1/8*105 and 53/91% in 108 conidia/ml was seen. Analyses of the effect of conidial suspension ranging from 104 to 108 conidia/ml and sterile distilled water as control on adult parasitoid indicated that mean mortality increased from %10/48 at 104 to %70/93 at 108 conidia/ml. The LC50 value after five days was 1/9*106 conidia/ml. These results indicated that we must careful in using DAOM 198499 at the same time with parasitoid. Fungus LC50 estimated for whitefly cause mortality on parasitoid, particularly when they were in larval stage.

Poster / Fungi. F-17

Stresses improve Beauveria bassiana efficacy for Tribolium castaneum

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Atmosphere modification is an increasingly common, chemical-free approach to microbe and insect control in grain storage and processing. Generally this refers to oxygen reduction and CO2 augmentation. In addition, desiccating conditions are common in stored products and are increased with aeration or atmosphere modification. I tested desiccation, oxygen reduction, and CO2 stress for there effects on Beauveria bassiana efficacy for red flour beetles. Exposure of beetle larvae to a sublethal reduction in oxygen to 5.0-5.3 % for three days significantly increased the B. bassiana- associated mortality. Two days of exposure to 40% CO2 also significantly increased mortality. Desiccation increased the mortality of both larvae and adults that were treated with fungus, even when the desiccation stress was withdrawn prior to fungal treatment. The transcription responses to the stresses and B. bassiana are now being evaluated with microarrays.

Poster / Fungi. F-18

Utility of fungicides to isolate invertebrate-pathogenic fungi

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Knowledge about isolation of entomopathogenic fungi with selective media is restricted to few species. We studied in vitro susceptibility of 41 invertebrate pathogens and 11 contaminants to dodine, benomyl, thiabendazole, manzate, cupric sulphate and copper oxychloride. Germination, formation of halo, colonies and new conidia on colonies by Beauveria bassiana, Evlachovaea sp, Metarhizium anisopliae and Tolypocladium cylindrosporum depended on an isolate-by-isolate basis on the type and concentration of incorporated fungicides. Most other pathogenic fungi and contaminants had clear patterns of susceptibility at benomyl (1 mg/l), dodine (50 mg/l), manzate (100 mg/l), cupric sulphate (200 mg/l) and thiabendazole (4 mg/l). Thiabendazole permitted development of many pathogenic fungi and inhibited most contaminants and seemed to be the most appropriate fungicide to isolate fungi pathogenic to invertebrates. Benomyl and dodine, increasingly unavailable, are widely used in selective media for Beauveria and Metarhizium and were also useful for other entomopathogenic fungi. Copper oxychloride, manzate and cupric sulphate are not recommended due to its low fungicidal activity. No fungicide was active against the fast-growing zygomycetes Cunninghamella echinulata, Mucor plumbeus and Rhizopus arrhizus. Results underline the need to adapt the choice and concentration of fungicide to the target fungus and the likely contaminants from specific habitats.

Poster / Fungi. F-19

Roll-method for mass-production of hyphomycetous fungi

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The market of mycopesticides presents formulations based on different groups of hyphomycetous entomopathogenic as well as antagonistic fungi. All these fungi form conidia superficially on solid substratum providing optimal aeration. There are two principal mass-production technologies of fungi guaranteeing these conditions: the first is a two stage technology based on submerged cultivation of fungi, and subsequent transfer of fungal biomass to flat-bottomed bathes to achieve sporulation; the second variant is based on the utilization of different solid nutrient substrata. We suggest an innovative technology of mass-production of hyphomycetous fungi. This technology is based on the utilization of double-layer rolls which offer a new possibility for effective production and application of mycopesticides. The double-layer roll comprises the use of hydroscopic paper or fabric as an inner layer, and an external layer bases on any material with an irregular surface and that will provide air ventilation inside the roll body (for example plastic bubble packing material). Fungal biomass obtained after submerged cultivation is uniformly distributed by spray along the internal layer. Inoculated rolls are incubated in optimal conditions in accordance with the biological properties of the specific fungus. This method of production of fungi offers the following advantages and improvements: a) productivity of fungi significantly increase in comparison with traditional two steps technology; b) process of production is shortened; c) area required for industrialization is reduced many times; d) industrial process can be easily mechanized and automatize. In addition this method provides new possibilities for formulation and application of fungal material.

Poster / Fungi. F-20

Efficiency of three different mass-production methods for hyphomycetous fungi

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Three different mass-production methods for hyphomycetous fungi including a two stage method, a solid substratum method (millet and sorghum) and a new roll method were tested in order to estimate the conidia productivity and processing duration of each technique. The entomopathogenic fungi Beauveria bassiana and Lecanicillium muscarium were used in the experiments. Initial biomass was prepared using submerged cultivation of fungi on Sabouraud dextrose with yeast extract medium. Fungal biomass was used for conidia production according to the three different technologies in analysis. For the two stage method the fungal biomass was transferred to 28 x 15 cm flat-bottomed bathes (400 ml and 200 ml biomass/bath) in order to reach sporulation. For the second method, 500 g of solid substratum were inoculated with 10 ml suspension (108 conidia/ml), after special processing in cultivation units, and then incubated for conidia formation. For the roll method a special tubular device was developed comprising a hydroscopic material as the inner layer and an external layer with an irregular surface (plastic bubble packing material). Both were assembled in order to allow air circulation inside the roll body. Fungal biomass obtained after submerged cultivation was uniformly distributed along the hydroscopic material. It was established that durability of technology for each method was 8±2 days (two stage method), 12±3 days (solid substratum method), and 6±1 days (roll method). The number of B. bassiana conidia harvested, per gram of nutrient substrata was 1.1-1.7 x 109 (two stage

method), 0.5-1.5 x 109 (solid substratum method), and 5.1 x 7.0 x 109 (roll method). For L. muscarium the number of conidia harvested ranged from 6.6-7.3 x 108, 1.0-2.5 x 109 and 3.4 - 4.5 x 109 accordingly.

Poster / Fungi. F-21

Importance of viability of Verticillium lecanii (Lecanicillium spp.) on the cucumber leaf surface.

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We reported that the selection of hybrid strains based on pathogenicity against Aphis gossypii and Trialeurodes vaporariorum and viability on cucumber leaf surface (Aiuchi et al., 2007). It was declared that 2aF43 has high pathogenicity against cotton aphid and whitefly, furthermore high viability under low humidity condition. In this study, we investigated the correlation between viability on the leaf surface and pathogenicity against pest insect, from the view point of epiphytic effect. Vertalec, Mycotal and 2aF43 were sprayed to ventral leaf of cucumber, and incubated at 25°C, 5.6%RH glasshouse. The strain viability was evaluated using the dilution plate method on 14, 21 and 28 days post inoculation (dpi), and bioassay against A. gossypii by leaf disk method was conducted. Vertalec showed low viability on the leaf, however high pathogenicity against cotton aphid at 14dpi. On the other hand, there were no infected aphids on the leaf treated with Vertalec on 21dpi. Hybrid strain 2aF43 showed high viability and high pathogenicity on all investigation. From these results, it was clarified that the strain inherent high pathogenicity might be important, furthermore such a control effect could persist on the leaf for a long term by highly epiphytic characteristics of the hybrid strain.

Poster / Fungi. F-22

Brief encounters or lasting relationship? Detecting and quantifying field persistence of introduced Beauveria bassiana GHA for emerald ash borer control by use of real-time PCR

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Accurate monitoring of an introduced, mass-released microbial control agent is critical in evaluating its efficacy and in designing application strategies for insect pest control. As part of our multi-year study on the development and use of the entomopathogenic fungus Beauveria bassiana against the emerald ash borer, a major invasive pest of ash trees, we are determining persistence of the fungus sprayed on ash trees and leached onto soil. Previous sampling methods have relied on colony counts and identification of representative colonies by use of microsatellite markers, methods that we found laborious and inadequate for sample comparisons. We recently developed a real-time PCR assay to detect and quantify B. bassiana GHA, the active ingredient in the mycoinsecticide utilized in our field studies. Real-time PCR primers and probe, based on a 445-bp DNA fragment unique to GHA, generated a 96-bp fragment that was also specific to this strain. We also developed a DNA extraction method to maximize accuracy of quantification from environmental samples. DNA from fungal spores in mixed samples was extracted using bead mill homogenization followed by purification of the crude extract using Sephadex-polyvinylpolypyrrolidone micro columns. Efficacy and sensitivity of assays were tested by comparing spore number estimates between pure and mixed samples.

WEDNESDAY PM

Poster / Fungi. F-23

Variations in UV-B-irradiation tolerance for Beauveria spp. isolates from different latitudes, hosts and substrates

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Beauveria bassiana is an important entomopathogenic fungus that is used worldwide as a biological control agent of arthropods. Efficient biocontrol of arthropods with fungi requires maintenance of viability and virulence of the fungal inoculum after field application. Solar radiation, particularly the UV-B wavelengths, is a major environmental factor that can negatively affect these traits in entomopathogenic fungi. A preliminary trial with three isolates of B. bassiana (Bb 19, CG 310 and CG 481) indicated that 2 hours of UV-B irradiance at 978 mW m-2 (total dose = 7.04 kJ m-2) allowed separation of isolates into low, medium or high UV-B tolerance. This dose, therefore, was selected as a single dose to compare UV-B tolerance among 60 Beauveria spp. isolates (53 B. bassiana, 3 B. amorpha, 1 B. brongniartii, 1 B. vermiconia, 1 B. velata and 1 Engyodontium albus =Beauveria alba). There was high variability in tolerance to UV-B radiation among the B. bassiana isolates, ranging from virtually zero tolerance (e.g. Bb 03) to almost 80% tolerance (e.g. CG 228). Germination of B. bassiana conidia was delayed following UV-B radiation. Conidia of the other species were very sensitive to UV-B, with relative-mean-percentage germination ranging from 1.6% [E. albus (UFPE 3138)] to 29.1% [B. amorpha (ARSEF 656)]. Isolates of B. bassiana originating from lower latitudes tended to have higher UV-B tolerances than isolates from higher latitudes. A similar analysis based on host type did not indicate a correlation. This study identified several B. bassiana isolates with relatively high UV-B tolerance; and these isolates may have promise as arthropod-control agents under solar-irradiated field conditions.

Poster / Fungi. F-24

Screening, identification and determination of functional parameters of photosensitizers with antifungal action

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The significantly increased prevalence of medical mycoses, due to growing numbers of immunocompromised individuals as well as the emergence of new species and antimycotic-resistant strains of pathogenic fungi, constitutes a serious public health problem. In such a scenario, the development of new fungus-control techniques is highly desirable. We have used an insect-pathogenic fungus (Metarhizium anisopliae) and a saprophyte (Aspergillus nidulans) as models for optimizing a light-based antimycotic system. Initially developed as an alternative therapeutic method for cancer, PDT (photodynamic therapy) is a process that involves the use of a photosensitizer that preferrably accumulates in the target-cells and that can be visible light-activated. The activation of the photosensitizer leads to the generation of reactive-oxygen species that kill cells by damaging lipids, proteins, and DNA. APDT (antimicrobial photodynamic therapy) can be used to control localized infections and kill pathogenic fungi in the environment. In this study, we investigated MB (methylene blue), TBO (toluidine blue), and nitrosyl ruthenium complexes as photosensitizers in photoactivation of M. anisopliae and A. nidulans conidia. We also tested parameters such

as photosensitizer concentration (1 to 400 µg mL-1), pre-incubation time with the photosensitizer (0 to 60 min.), and dose (90 or 180 kJ m-2) of visible-light irradiance (50 W m-2). MB and TBO photoinactivated the conidia of both fungal species, with inactivation close to 100% when the appropriate combination of photosensitizer concentration and light-exposure time was used. In the dark, neither dye inactivated conidia of either species. Varying the pre-incubation time with the photosensitizers (MB and TBO) did not alter photoinactivation levels. Conidial inactivation with MB and TBO was higher with 60-minute light exposure than with 30-min treatments. Green and yellow conidia were less photoinactivated than mutants with white and violet conidia, indicating that conidial pigmentation influenced photosensitization. Nitrosyl ruthenium [Ru(NH. NHq)(tpy)NO](PF6)3] was not capable of photoinactivating conidia of either species. With M. anisopliae conidia, photoinactivation with MB and TBO did not occur when photosensitization was conducted in PDB medium (potato dextrose broth).

Poster / Fungi. F-25

Visible light during growth increases tolerance of Metarhizium anisopliae var. anisopliae conidia to UV-B radiation, but does not alter heat tolerance nor conidial yield

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Light conditions during culture are known to influence metabolism, growth, sexual and asexual development, and/or pigment formation in many fungi. In this study, conidia of Metarhizium anisopliae var. anisopliae (ARSEF 2575) were produced on PDAY (potato dextrose agar plus yeast extract) medium under continuous light or dark conditions. For comparison, conidia also were produced on minimal medium (MM) (=Czapek medium without saccharose) under continuous-dark incubation, which is known to produce conidia with high tolerance to heat and UV-B radiation (JIP, 2006, 93: 127-134). Conidia produced with continuous visible-light exposure during growth demonstrated significantly increased conidial tolerance to UV-B radiation. The UV-B tolerances of conidia produced on PDAY under continuous visible light (provided by two 15 W cool white Sylvania® fluorescent lamps, intensity approximately 12 W m-2) was similar to that of conidia produced on MM in the dark. Conidia produced on PDAY under continuous light conditions or on MM in the dark had two-fold higher UV-B tolerances than conidia produced on PDAY medium under dark conditions. Continuous light, however, did not significantly increase heat tolerance; viz. the heat tolerance of conidia produced under continuous light was statistically similar to that of conidia produced on MM and PDAY in the dark. Sporulation in many fungi is unaffected by light; in others, however, light is important for conidiogenesis. Continuous light is reported to increase conidial production of entomopathogenic fungi, including some strains of Metarhizium spp. In the current study, conidial production (yield) of M. anisopliae ARSEF 2575 on PDAY medium was similar with incubation under continuous-dark or continuous-light conditions.

Poster / Fungi. F-26

Harvesting of Insecticidal Chitinase produced from Entomopathogenic fungi, Beauveria bassiana DBB2507 using Enzyme Absorption Method

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Entomopathogenic fungi, Beauveria bassiana DBB2507 was isolated from soil in Korea. In vitro condition the supernatant among conidia, blastospores and supernatant showed the highest insecticidal activity about 88.9% of efficacy against Myzus persicae adults at 3 days after the treatment. The efficacy of non-autocleaved, autocleaved and protein-precipitated pallet using ammonium sulfate were 87.7%, 11.0% and 94.1%, respectively which means the main insecticidal components of supernatant could be proteins. Three kinds of enzymes, chitinase, Pr1 protease and Pr2 protease of supernatant were analyzed. Supernatant mainly hydrolyzed the substrates specific for chitinase, about 9.148 mM p-nitrophenol per hour. To acquire powder type of chitinase, several kinds of absorbent were tested for the precipitation of soluble chitinase because liquid type of chitinase is susceptible to thermostress. Among them skim milk showed the highest absorbing activity, about 90.5% of precipitation rate. Freeze-dried pallet diluted by 5,000 fold showed 92.9% of efficacy against Myzus persicae adults in laboratory bioassay. Chitinase stability of freeze-dried pellet was more stable about 82.3% than that of supernatant about 8.0% at 50°C for 2 hours.

Poster / Fungi. F-27

Induction of apoptosis in Sf-21 cell line by cultured fluid of the entomopathogenic fungus, Nomuraea rileyi

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The apoptosis in sf-21 cell line could be induced by cultured fluid of the entomopathogenic fungus, Nomuraea rileyi, based on changes in cell morphology and the onset of DNA laddering. We further observed DNA fragmentation in sf-21 cells using TUNEL staining. The level of apoptosis induced by N. rileyi cultured fluid was found to be dose-dependent. Furthermore, the induction of apoptosis in sf-21 cells was inhibited by adding the inhibitor of effector caspase, viz., z-DEVD-fmk, to the cultured fluid, indicating that sf-caspase-1 is involved in this apoptosis. Similarly, the inhibitor of initiator caspase, viz., z-VAD-fmk, was also inhibitory to the apoptosis. Therefore, both initiator and effector caspases are possibly involved in the apoptosis of sf-21 cells. In addition, we detected caspase-9 activity in the process of sf-21 apoptosis, suggesting that the initiator caspase in sf-21 is similar to that in mammalian cells.

Poster / Fungi. F-28

Tyrosine betaine: a new biomolecule isolated from Metarhizium anisopliae conidia

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Conidia are responsible for the reproduction, dispersal and environmental persistence of different fungal species of medical, industrial and agricultural interest. In pathogenic species, conidia are also the structures predominantly responsible for host infection. Natural fungal populations are controlled by environmental factors, such as solar radiation, temperature, and hyperosmotic stress caused by desiccation. These factors limit the survival and dispersal of pathogenic species and represent serious obstacles in the use of fungi like Metarhizium anisopliae as bioinsecticides. Several compounds present in fungal conidia, such as pigments, micosporines, sugars, amino acids, and polyols, have been associated with stress tolerance. The discovery of new molecules involved in conidial tolerance to stress-inducing environmental factors will contribute both to 1) better understanding of the persistence, dispersal and germination

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of pathogenic species in the environment, and 2) the development of entomopathogenic fungal strains with increased stress resistance for use in biological control of insect pests. Extracts of M. anisopliae var anisopliae were examined by 1H and 13C nuclear magnetic resonance spectroscopy and mass spectrometry for the presence of stress protectants. A compound whose natural occurrence was never described before was discovered. It consists of betaine conjugated with tyrosine which was identified as 2-{[1-carboxy-2-(4-hidroxyph enyl)ethyl]amino}-N,N,N-trimethyl-2-oxoetanammoniun (chemical formula: C14H21N2O4+; mass 281.1496 and maximum absorbance at 275 nm). Glicine betaine and proline betaine act as osmoprotectants in animals, plants and bacteria. Tryptophan betaine product by ectomycorrhizal fungi is important in microbe-plant interaction. The discovery of tyrosine betaine biological functions in M. anisopliae and the search for biotechnological applications for it are providing interesting intellectual and practical challenges.

Poster / Fungi. F-29

Genotypic variability among Brazilian isolates of Beauveria bassiana

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The genetic variability among B. bassiana isolates was investigated to provide fundamental biological information and to help satisfy the requirements for biopesticide registration. Accurate identification of isolates is important in monitoring the persistence and behaviour of an isolate following its release into the environment. The present study used AFLP, and ITS1 and ITS2 sequencing to characterize 50 Brazilian B. bassiana isolates originating from various geographic regions, arthropods hosts or substrates. Overall, the several isolates demonstrated significant genetic variation. Moreover, the genetic distance among B. bassiana isolates was associated with the geographical distances of their origins. The level of genetic variation observed among these Brazilian isolates is in agreement with previous studies of B. bassiana from other regions, and indicates that B. bassiana represents a species aggregate. In addition, the high similarity coefficient of certain isolates from cattle ticks (Boophilus microplus) from a somewhat localized geographic area (Rio de Janeiro and Sao Paulo States) provided evidence of clonal structure. The genetic distances among B. bassiana isolates were not correlated with host of origin.

Poster / Fungi. F-30

Identification of Metarhizium anisopliae transcripts expressed during the fungus- insect interaction

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The identification of genes contributing to the establishment and disease progression of entomopathogenic fungi within their insect hosts has been conducted to date largely using in vitro systems mimicking specific phases of the infection. We are exploring the use of in vivo techniques to identify fungal genes expressed during pathogenicity and involved in secondary metabolism using Metarhizium anisopliae and Spodoptera exigua as our model systems. One hypothesis-driven approach is to evaluate expression in vivo by RT-PCR of genes predicted to be involved in fungal secondary metabolism. Another unbiased approach is focused on developing cDNA enriched for fungal transcripts expressed during the infection process by using either suppression subtractive hybridization (SSH) or a modification of representational difference analysis (RDA). In both cases, tester cDNA was prepared from a pool of RNAs extracted from S. exigua larvae at multiple time points after inoculation with M. anisopliae. Driver cDNA was prepared from mock-inoculated S. exigua larvae collected at identical time points as for the tester cDNA. Subtraction/enrichment efficiency was tested using semi-quantitative PCR and revealed marked differences between the two techniques. These methods will be useful for the generation of pathogenicity-related cDNA libraries containing both fungal and insect-responsive transcripts.

NEMATODES

Poster / Nematodes. N-01

The host-parasite biology of Frankliniella fusca (Hinds) (Thysanoptera: Thripidae) and Thripinema fuscum Tipping & Nguyen (Tylenchida: Allantonematidae)

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The tobacco thrips, Frankliniella fusca (Hinds) (Thysanoptera: Thripidae), is a polyphagous insect pest that feeds on agriculturally important plants in the eastern United States, Mexico, and Canada. It is one of ten known thrips species capable of transmitting Tomato spotted wilt virus (Bunyaviridae: Tospovirus) in vegetable crops such as peanut, tobacco, tomato, pepper, as well as in numerous ornamentals and grasses. The discovery of the entomogenous nematode Thripinema fuscum Tipping & Nguyen (Tylenchida: Allantonematidae) parasitizing natural populations of F. fusca suggests it may be used as a biological control agent. This parasite reduces feeding and fecundity of F. fusca with negligible effects on host longevity and mortality, in turn, reducing TSWV incidence in field conditions. Light and electron microscopy have been used to examine the in vivo development of T. fuscum and the subsequent disruption to host tissues following parasitization. Special emphasis has been directed at describing the ultrastructure of the parasitic nematode as well as the salivary glands and reproductive tissues of F. fusca. The mechanisms regulating the ability of these parasites to shut down fecundity and to interfere with viral competency of insect vectors will be discussed.

Poster / Nematodes. N-02

Stable association between Serratia marcescens and Steinernema carpocapsae during the infection process.

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The strain LBIN-1 of Steinernema carpocapsae was isolated in Tapachula, Chis., Mexico, from highly white grub infested maize soil, and identified by ITS sequence. Amplification of the strain in Galleria mellonella larvae showed a distinctive red coloration. Red bacterial colonies were isolated from larval suspensions and 16S sequencing identified it as Serratia marcescens. After several generations in the laboratory, the red coloration of larvae was significantly reduced. IJs of LBIN-1 submerged in a S. marcescens suspension restored the red coloration. However, when larvae were infected with non-inoculated IJs, suspensions still showed the presence of S. marcescens, but at reduced numbers. S. marcescens showed no infectivity when tested separately. When IJs were externally sterilized and used to infect larvae, no S. marcescens was detected, either

from the larval suspensions or from treated IJ suspensions. Furthermore, up to now, 25 generations have been kept in the laboratory, and S. marcescens is still being detected in larval suspensions. These results indicate that IJs have the ability to efficiently transmit the bacterium; that the association remains through generation, although at reduced numbers; that the nematode is required for S. marcescens to infect the larvae; and that the bacterium is externally associated to the nematode.

Poster / Nematodes. N-03

To protect and enhance conservation and sustainable use of the entomopathogenic nematode biodiversity of Chile

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A Darwin Initiative project, administered by the UK Department for Environment, Food and Rural Affairs, was established in 2006 to create a culture collection in Chile of indigenous entomopathogenic nematodes (epn) and to build on the expertise required to curate and profile them. The long-term objective of the work is to develop biological control agents (biopesticides) based on epn. Chile is no exception in an international push to reduce chemical pesticides on farms and biopesticides can form key components of a more IPM approach. Chile stretches for over 2,700 miles, bordered on the West by the Pacific Ocean and on the East by the Andes. Ecological conditions cover extremes from near Antarctic in the South through to desert in the North, with marked climatic and habitat differences. Collaborating partners on the project, CABI and INIA, have selected six survey sites, covering major ecological habitats in Chile as a function of climate, vegetation, topography and soil type. Locally obtained epn will be profiled and their respective ecological habitats examined to identify links between pathogen and habitat. Two sites will be surveyed every year. Sites in the South and the far North have recently been surveyed; the samples are presently being processed.

Poster / Nematodes. N-04

Diversity of entomopathogenic Nematodes (Steinernematidae, Heterorhabditidae) in Jordan

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Until now, only a few systematic surveys of entomopathogenic nematodes (EPN) have been conducted in Middle Eastern countries. Many of the recovered EPN species in this region have shown to own distinctive qualities that enable their survival in unique environments, such as high temperatures and low moisture levels tolerance. These new species and strains, with unique environmental tolerances, are more suitable for their consideration in pest management programs in xerophitic regions. With this background in mind, we recently conducted a survey of EPN in Jordan. This study provides the first record of the diversity and distribution of these nematodes in this country. Jordan's three geographic regions: 1) the highlands, 2) Jordan valley and 3) the desert region were sampled. Within each region, natural habitats and agricultural areas characteristic to each region were considered for sampling purposes. Five EPN species including four Steinernema and one Heterorhabditis, were recovered. Nematodes were identified using a combination of molecular markers and classic morphological diagnostic tools. Abiotic characteristics such as soil type, annual rainfall and elevation were also recorded. The abundance and distribution of EPN spp. in Jordan will be presented and discussed in relation to these parameters.

Poster / Nematodes. N-05

N-acetyl β-D-glucosaminidase, a cuticle enzyme secreted by axenic Steinernema carpocapsae suppresses the immune system of the Greater wax moth, Galleria mellonella

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The nematode-bacterium complex, Steinernema carpocapsae-Xenorhabdus nematophila, is a virulent insect pathogenic system for larvae of the pest insect, the Greater wax moth, Galleria mellonella. Upon reaching the lepidopteran's hemocoel, there is a period during which the nematode must avoid initiating the insect's non-self humoral and cellular systems to ensure subsequent bacterial release. Immunosuppressive and evasive activities have been associated with steinernematid cuticle. However axenic S. carpocapsae are known to release trypsin-like and chymotrypsin-like enzymes, which partially limit hemocyte responses to foreign antigens implying other factors may be involved. Herein an APIZYM enzyme analysis of axenic exudate revealed a negative correlation with N-acetyl β-D-glucosaminidase (NAG) activity and hemocyte adhesion to slides. Confirmation of NAG as an immunosuppressant was based on blocking the inhibition of hemocyte attachment to glass slides by the exudate and commercial NAG sources using the NAG inhibitors α-D-mannose and p-nitrophenyl-α-D-mannopyranoside. Glucose and trehalose, at physiological levels in G. mellonella, did not impair NAG activity of either the exudate or commercial source; precluding these plasma sugars limiting immunosuppression by the nematode. The immunosuppressive activities of NAG suggests it is a virulence factor of the entomopathogenic nematode, Steinernema carpocapsae.

Poster / Nematodes. N-06

Blackfly mermithids from Québec : an ecological and molecular study

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Mermithids are well known parasites of blackflies. Among environmental parameters (pH, current speed...), only the stream depth was found to matter with respect to the presence of mermithids. We initially identified 4 morphological species, Mesomermis flumenalis, M. camdenensis, Gastromermis viridis and Isomermis wisconsinensis, each having a typical seasonal and host profile. Given the inadequacy of current morphological keys and the frequent occurrence of damaged specimens, consequently many individuals could not be properly characterized at first. Accordingly, we developed a molecular typing assay based on the amplification of nuclear 18S rDNA and mitochondrial COI markers. Using species-targeted primers, we easily distinguish between the 4 previous species, without any interference from contaminating blackfly material. Our molecular method allows the easy identification of juvenile or damaged specimens. Furthermore, in the cases of multiparasitism, the molecular markers identify 2 mermithids species parasitizing the same blackfly larva. Also, sequence data show that M. flumenalis consists in fact of 3 distinct species, namely summer and winter variants in addition to a yet undescribed species. 18S rDNA and COI-derived trees, with Caenorhabditis elegans as the outgroup sequence, indicate for mermithids a monophyletic origin, including a closed cluster containing all 4 sequences from the Mesomermis genus.

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Poster / Nematodes. N-07

Detection and sequence analysis of insecticidal gene tccC1/ xptB1 homologues from Xenorhabdus nematophilus (CR5), bacterial symbiont of the entomophagous nematode Steinernema westerii (Rhabditida: Steinernematidae) isolated from northern Costa Rica.

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We have identified and sequenced a gene up to 99% homologous to tccC1 from a bacterial strain (CR5) isolated from Steinernema websterii recovered from northern Costa Rica. This bacterial symbiont was identify as Xenorhabdus nematophilus (RIOBRAVIS) based on biochemical and 16S ribosomal RNA sequencing analysis (96% homology). To evaluate the existence of the gene tccC1/xptB1, presumably the active delivered insecticidal product of the toxin complex of X. nemathophilus, we carried out PCR amplifications in extracted genomic DNA from CR5 strain, using appropriate primers described elsewhere for core (180 pb) and extension regions [fragments 1 (674 pb), 2 (1062 pb), 3 (875 pb) and 4 (495 pb)].

Through the sequencing analysis, core region -known to be highly conserved- showed homology with up to 100% identity with Genebank accession No AY538173, corresponding to insecticidal gene tccC1/xptB from X. nematophilus strain isolated from Korean entomophagous nematode Steinernema glaseri MK. Meanwhile, the remaining fragments showed about 99% homology with the same accession. Additionally, five single nucleotide polymorphisms (SNIPS) where identified in fragments 1 (nucleotides at positions 537, 549 and 630) and 2 (nucleotides at positions 402 and 438).

Further analysis will include long PCR amplification, cloning and heterologous expression of this gene.

Poster / Nematodes. N-08

Investigation on biological control of alfalfa stem nematode (Ditylenchus dipsaci) by using Rhizoglyphus robini mite

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The pot experient was used to apply the R .robini mite for biological control of alfalfa stem nematode D.dipsaci in soil.the experiment was performed via random blocked plan. The average of 3replicates was used for each treatment. First treatment included 500gr sterilized soil, four alfalfa seedling pods(three cutting Hamedan),one thousand nematodes(D.dipsaci) and one hundred mites(R.robini).Second treatment comprised of 500gr sterilized soil,four alfalfa seedling pods(three cutting Hamedan) and one thousand nematodes(D.dipsaci).Last treatment contained 500gr sterilized soil and four alfalfa seedling(three cutting Hamedan).All of these treatments were studied within 3 months at average temperature of 27C and 85±5c/o relative humidity under greenhouse effect. It was revealed that R.robini mite controlled 37c/o of D.dipsaci population. The wet weight of alfalfa seedling pods for versatile treatments showed a meaningful difference with each other. It was concluded that although, R.robini is one of the most important pestes of onion,potatoe and stored ornamental plant bulbs, but it can play a major and effective role in integrated pest management plans against alfalfa stem nematode (Ditylenchus dipsaci).

Poster / Nematodes. N-09

Managing chickpea pod borer, Helicoverpa armigera (Hübner) with Heterorhabditis indica – A success story

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Chickpea, an important pulse crop of India occupies an area of 6.68 m ha with a total production of 5.07 m tones. The pod borer, Helicoverpa armigera (Hübner) is a major pest causing as high as 95% damage (Sachan and Katti, 1994). Wide spread appearance of resistance to chemical insecticides including widely used pyrethroids in late 1980's caused an increase in losses due to this pest and has made control by chemical increasingly unreliable and expensive (Armes et. al., 1992).

Entomopathogenic nematodes (EPNs) in families Steinernematidae and Heterorhabditidae have considerable potential to control several insect pests (Gaugler and Kaya, 1999). A native species, Heterorhabitis indica (Poinar et al., 1992) from India has great potential in controlling several crop pests including H. armigera (Karunakar et al., 2002). It has also been shown that the performance of EPNs can be enhanced by integrating with other entomopathogens and botanicals (Choo et al., 1998). Hence, a series of laboratory and field studies were undertaken to develop bio-intensive management strategy against H. armigera by integrating locally isolated H. indica (RCR) strain with selective entomopathogens and botanicals in chickpea ecosystem during 2002-2005.

Poster / Nematodes. N-10

Metabolites from axenic Steinernema carpocapsae suppress the non-self responses of the pest insect Galleria mellonella

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The insect pathogenic nematode, S. carpocapsae develops in the blood of insects without being attacked by the insect blood cells. Since the nematodes release metabolites (exudates) into the hemolymph, we hypothesized that the exudates suppress the non-self responses of hemocytes to glass slides and bacteria Exudates from live axenic nematodes in phosphate-buffered saline elevated the total viable hemocyte counts and granular cell levels by 8 h post-injection while exudate from dead nematodes had no effect on the hemocytes. The increase in hemocyte may represent hemocyte dissociation from tissues since the exudate dissociated the granular cells from slides but not plasmatocytes. Much of the immunosuppressing activity of the exudate was heat labile (destroyed at 65oC). A strong positive correlation was observed between the level of chymotrypsin and trypsin substrates hydrolysis and the level of adhering total hemocytes. Treatment of the exudate with both chymotrypsin and trypsin specific inhibitors (metabolic inhibitors and antibodies) lowered hemocyte binding to slides and suppressed the removal of Bacillus subtilis and Xenorhabdus nematophila from G. mellonella hemocytes in vivo. Release of chymotrypsin and trypsin from nematodes was detected by 2 h with a peak at 4 h for trypsin and a peak at 6h for chymotrypsin. Nematode exudate prevents hemocyte attachment, enhances dissociation of hemocytes from tissues and slides and suppresses removal of bacteria from hemocytes.

Poster / Nematodes. N-11

Some anti-nematodes compounds as candidates for a trunk-injection agent against the pine wilt disease

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It is well known that pine wilt disease in a number of Pinus species is caused by pine wood nematode, Bursaphelenchus xylophilus (Steiner & Buhrer) Nickle, transmitted by the pine sawyer, Monochamus alternatus Hope. The present methods of preventing the disease are based on killing the vector by spraying chemical insecticides, or controlling the pathogen by injecting the three trunks with an anti-nematode compounds. Although some anti-nematodes compounds, such as mesulfenfos, morantel tartrate and levamisol hydrochloride, are used as effective agents but they care expensive. This study aimed to develop more effective and cheaper trunk-injection agents against pine wood nematode. We examined the inhibition rate of four anti-nematodes agents viz abamectin 1.8% EC, emamectin benzoate 2.15% EC, fosthiazate 30% SL, imidacloprid 4% SL, and morantel tartrate, against B. xylophilus on potato dextrose agar medium. This compounds resulted in an inhibition rate of nematodes agents with over than 99.9% with exception of imidacloprid. In the field experiment, anti-nematodes candidates, abamectin, emamectin benzoate were injected into pine trees. After 2 months, the pine wood nematode were infected into pine trees. Consequently the prevention effect of these candidates was 96.7%. The artificial damaged rate by pine wood nematode only 76.7% and the native damaged rate by pine wood nematode was 11.6% in the current year after experiment and the next year was 4.0%, but abamectin and emamectin benzoate was below than 0.3%.

Poster / Nematodes. N-12

Biological control of root knot nematode, Meloidogyne hapla using plant extracts

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Thirty two plant species were selected for environmental friendly control of root-knot nematode in the soil which is becoming a problem for in crops cultures in Korea. Authorized nematicidal activity against Meloidogyne hapla through bioassay laboratory after refined preparation of plant materials by four extractions method (methanol, hexane, hot and cold). Mortality rates of the nematodes with the methods of methanol or hexane extracts derived from plants at 1,000 ppm after 24 hr treatment to Meloidogyne hapla in the laboratory were recorded. Nematicidal activities of the methanol or hexane extracts (1,000 ppm) in Daphne genkwa leaf, Eugenia Caryophyllata flower, Quisqualis indica fruit and Zingiber officinale rhizome were more than 80%. At a concentration of 500 ppm, the extracts of the D. genkwa and Q. indica showed more then 95% mortality rates, Z. officinale and Eugenia Caryophyllata extracts showed more then 90% mortality rate to M. hapla. Nematicidal activities of the hot water extracts at 1,000 ppm in Z. officinale and Q. indica were 98.3% and 97.1%, respectively. Q. indica extract showed high nematicidal activity to M. hapla at all extraction methods. At 1,000 ppm, high control rate against M. hapla was observed for the hexane extract of Q. indica in tomato planted pot. From these results, Z. officinale and Q. indica extracts could be used as environmental friendly control against M. hapla.

Poster / Nematodes. N-13

Biocontrol of Meloidogyne incognita juveniles by Plant Extracts

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Twenty three different medicinal plant species (trees and herbs) were collected from Sudan, mostly from Gezira province. Methanol extract of 29 samples including different plant portions (stems, leaves, fruits and seeds) of the 23 plant species representing 19 plant genera were screened in the laboratory against juveniles of Meloidogyne incognita. 500 ppm were first used from all the plant extracts for 24, 48 and 72 hours time of exposure. However, among these, 7 plant samples gave mortality rate between 80-98 % at 72 hours time with the extracts of Dinebra retrofkexa (leaves) Cucumus mello var. agrestis (fruits), Ziziphus spina-christ (leaves), Acacia nilotica (pods), Chenopodium album (leaves), Sonchus cornatus (leaves) and Caliotropis prcocera (leaves). While 8 samples gave mortality rate between 50-70 %, whereas, the rest gave mortality between 0-49% at the same time of exposure. The best 7 Plant extracts above were further screened against the same nematode species juveniles using 50 ppm as well for 24, 48, and 72 hours time of exposure. Two extracts gave mortality rates of 82 and 75 % representing the extracts of Accacia nilotica and Dinebra retroflexa respectively. While the other 5 extracts gave mortality between 24-68 % mortality.

MICROSPORIDIA

Poster / Microsporidia. MS-02

The influence of seasonality and red imported fire ant (Solenopsis invicta) caste and colony social form on the prevalence and spore titer of the microsporidium Thelohania solenopsae.

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The microsporidium Thelohania solenopsae is a naturally occurring entomopathogen that shows potential for being developed as a biological control agent against the red imported fire ant, Solenopsis invicta. T. solenopsae has been shown to adversely affect the survival of workers and queens as well as the fecundity of S. invicta queens in the laboratory. There are, however, limited data on the field biology and epizootiology of this microsprodium. For example, the persistence of the disease across years and the influence of seasonality on the prevalence of the infection and spore titers are poorly understood. The distribution of microsporidiosis and spore counts across the different ant castes have not been studied extensively either. Fire ant social form is well accepted as a key determinant of T. solenopsae infections. Yet, it is not known why the disease is reported more frequently in polygyne than monogyne S. invicta. In this study, we examine the effect of seasonality on the prevalence of the disease and spore counts as well as the multi-year persistence of T. solenopsae infections at multiple sites in Louisiana; we document the concentration and type of T. solenopsae spores found in different S. invicta castes; and, finally, we compare spore levels in the two S. invicta social forms in an attempt to explain why the disease is observed more commonly in polygyne ants.

Poster / Microsporidia. MS-03

Effects of a novel microsporidian isolate from Poland on larvae of the Gypsy Moth (Lymantria dispar L.)

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Over the last decade, several microsporidia have been isolated from European populations of the gypsy moth (Lymantria dispar) and tested in laboratory for their potential in biological control. In 2006, a novel microsporidian was found in a population of gypsy moth (GM) feeding on willow in eastern Poland. A first characterization places this isolate (Accession No.1997-D, INHS Collection) in the genus Nosema. Other studies have shown that different Nosema –isolates show very diverse interaction with the host insect, ranging from very moderate to highly virulent.

The effects of the newly discovered isolate were tested in laboratory experiments. Third instar larvae of GM were infected with dosages from 200 to 50,000 spores/µl and reared individually to monitor the influence of the infection on larval mortality, development, weight, pupation and eclosion rates. Data were recorded twice per day. Furthermore, the number of oviposited eggs laid by infected and healthy GM and the hatch rates of the first instars were determined.

The infection with Nosema sp. delayed the larval development and reduced the larval and pupal weights, as well as the survival rates of larvae und pupae. The mortality occurred through all development stages and was dose-dependant. Few larvae fed with the highest dose survived to the adult stage. The infection of one or both of the mated adults caused a reduction of the number of oviposited eggs. This effect was most obvious in infected females. The hatch rate of first instars varied considerably. Results on vertical transmission will be presented. The results will be compared to similar studies of other microsporidian isolates and the potential of the Polish isolate in the regulation of GM populations will be discussed.

Poster / Microsporidia. MS-05

A new Vairimorpha isolate from Ocinara lida in Taiwan

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A microsporidium was isolated from the larvae of Ocinara lida in Taiwan. This isolate was found predominantly in fat body tissues, different from a previously isolated microsporidium, Endoreticulatus sp, which parasitized the midgut cells. The mature spores of the new isolate are diplokaryotic and the size averages 4.08 + 0.22 x 2.15 + 0.16 µm (length x width). Octospores were also observed in tissue smears of the infected larvae. The small subunit rRNA (SSUrRNA) gene consists of 1,248 bp and 36.86% GC content. The nucleotide identities between this isolate and other members of Vairimorpha complex are from 95% to 99%. Identities to the members of Nosema complex are 81%~83%. Phylogenetic analysis based on the SSUrRNA sequences placed this isolate in the Vairimorpha complex. We concluded that this isolate is a member of the genus Vairimorpha, but we need more molecular and morphological data to confirm that the isolate is a new species.

Poster / Microsporidia. MS-06

Molecular Data and Phylogeny of Nosema infecting Lepidopteran Forest Defoliators in the Genus Choristoneura and Malacosoma

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Nosema isolates from 5 lepidopteran forest defoliators; Nosema fumiferanae, Nosema sp. (Choristoneura pinus pinus, CPP), Nosema sp. (Choristoneura, occidentalis, CO) Nosema thomsoni and Nosema disstriae were compared by using sequence data derived from their small subunit (SSU) rRNA genes. N. thomsoni shared only ~ 82 % SSU rRNA sequence identity with all the described Nosema species and there was no substantial genetic variability (0.6%-1.5%) among the SSU rRNA sequences of N. fumiferanae, Nosema sp. CPP, Nosema sp. CO and the sister taxon N. disstriae. We determined that N. fumiferanae, Nosema sp. CPP, Nosema sp. CO and N. disstriae have a reverse arrangement in the rRNA gene (LSU-ITS-SSU) while N. thomsoni has the typical arrangement (SSU-ITS-LSU). Although the mechanism for rearrangement of the rRNA gene subunits is not known we provide a possible explanation for the localization. The SSU rRNA sequences and data from other microsporidian species were used to infer a phylogenetic tree. Nosema fumiferanae, Nosema sp. CPP and Nosema sp. CO clustered together and appeared to be closely related. The higher sequence similarities, the reverse arrangement in the rRNA gene subunits and the phylogenetic clustering suggest that these three parasites belong to the same species.

VIRUSES II

Poster / Viruses. V-26

Development of a direct cloning system for the baculovirus, Anticarsia gemmatalis Multiple Nucleopolyhedrovirus (AgMNPV).

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The AgMNPV virus has been one of the great successes of microbial-based insect control of the velvetbean caterpillar in South America. We developed a baculovirus direct cloning system based on homing endonucleases (HENs) which we called the "Homingbac system". This system allows the direct cloning of gene cassettes into baculovirus genomes without the need for bacteria or bacteriophages. Presently we have been developing a new generation of the Homingbac system in AgMNPV. This "Ag-Homingbac" includes a β-glucuronidase (GUS) selectable marker which is excised from new recombinants. As with our other Homingbac baculoviruses, we have engineered the Homingbac system components into the egt locus such that the polyhedra phenotype and per os infectivity are retained. We have also made several improvements to the Homingbac system. Firstly, we use two different HEN cloning sites to ensure that HEN-cut virus DNA does not ligate unless a foreign gene is inserted. Secondly, a virus promoter was placed outside of the HEN cloning sites such that exogenous ORFs may be cloned directly. This feature potentially enables the cloning of cDNA expression libraries into AgMNPV. It is expected that this Ag-Homingbac cloning system will be a valuable new tool for bioprospecting for insecticidal genes.

Poster / Viruses. V-27

A two color tag system to study virus-virus interaction in vitro.

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Mixed infection of nucleopolyhedroviruses in insects is common in natural environment. How two or more nucleopolyhedroviruses interact in insects or in cell lines is difficult to study. We developed a two color tag system which can be used to study virus-virus and virus-cell interaction more easily. We engineered the Autographa californica NPV (AcMNPV) by inserting the red fluorescent protein gene (RFP) downstream of the polyhedrin promoter in the polyhe-

drin locus (AcRed). We also inserted the green fluorescent protein (GFP) gene downstream of the polyhedrin promoter in the gp37 locus and p10 locus in narrow host-range Thysanoplusia orichalcea nucleopolyhedrovirus (ThGFP) and Spodoptera exigua nucleopolyhedrovirus (SeGFP), respectively. Choristoneura fumiferana NPV (CfGFP) containing the gfp gene in the egt locus was also included in this study. We performed coinfection of AcRed/ThGFP, AcRed/CfGFP and AcRed/SeGFP in Sf-21 and Hi5 cells. Individual virus cell infection was also performed. Individual virus infection revealed that ThGFP, CfGFP and SeGFP showed 1-3% of Sf21cells with GFP expression. Moreover, SeGFP also showed about 1% cell infection in Hi5. However, when coinfected with AcRed, ThGFP and SeGFP infection increased 20 folds in Sf21and Hi5 cells, respectively. No enhancement by AcRed was found in Sf-21 cells coinfected by CfGFP or SeGFP.

Poster / Viruses. V-28

To study the insecticidal efficacy of Spodoptera exigua multiple nucleopolyhedrovirus combined with wheat germ agglutinin or concanavalin A

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In this study, we attempted to demonstrate the enhanced insecticide activity of wheat germ agglutinin (WGA) and Concanavalin A (ConA) on Spodoptera exigua multiple nucleopolyhedrovirus (SeMNPV). Results show that the combination of SeMNPV with 0.2, 0.5 and 1% WGA caused increases of 42, 47 and 57% mortality of 2nd instar S. exigua at 4 days post infection, respectively. On the other side, the combination of SeMNPV with 0.2, 0.5 and 1% ConA caused increases of 30, 35 and 57% mortality of 2nd instar S. exigua at 3 days post infection, respectively. For 3rd instar S. exigua, the combination of SeMNPV with 0.5, 1% WGA or 0.2, 0.5, 1% ConA caused increases about 20% mortality at 6 days post infection. In addition, the different concentration of WGA and ConA combined with SeMNPV did not significantly caused different mortality from that of 4th instar S. exigua inoculated with SeMNPV alone. Our results also indicate that 1%WGA or ConA combined with SeMNPV has a significant increase the insecticidal potency on 2nd instar S. exigua, they showed the ET50 and EC50 were dramatic reduced than treated with SeMNPV alone, LT50 value was reduced from 4.05 days to 3.13 and 2.34 days; EC50 value was reduced from 1.46 x105 PIBs/ml to 6.35 x104 and 2.11 x104 PIBs/ml, respectively. Similarly treatment on 3rd instar S. exigua, LT50 value was reduced from 4.47 days to 3.96 and 3.94 days; EC50 value was reduced from 5.48 x105 PIBs/ml to 2.35 x105 and 2.23 x105 PIBs/ml, respectively. Thus, the results have been illustrated that 1% WGA and ConA can significantly enhanced the insecticide activity of SeMNPV, which is of actually applicative value in future.

Poster / Viruses. V-29

Molecular techniques for the detection, differentiation and quantitation of baculovirus isolates in Choristoneura fumiferana

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A multiplex primer set was used to rapidly detect and differentiate between; CfMNPV, CfDEFNPV, and other baculoviral DNA sequences in overtly infected C. fumiferana to a detection limit of less than 7000 genomes. A more sensitive nested PCR system was optimized to identify baculoviral DNA sequences in persistently infected larvae, and from crude genomic DNA extractions, with a detection limit of less than 70 genomes. The system was adapted to an SSCP protocol to differentiate baculovirus species and potentially genotypic variants. Persistent baculoviral infections could be detected in around 15% of adults in both diapausing and non-diapausing C. fumiferana stock. A real-time PCR protocol is under development for the quantitation of CfMNPV and CfDEFNPV in mixed inoculations. CfDEFNPV appears to have a synergistic effect on total mortality at low doses, but an inhibitory effect at higher doses. The suite of molecular analyses developed will be used to dissect the proportion of larval mortality attributable to each isolate, and relative viral load in individual larvae, and to monitor levels of recombination and generation of sequence variation. These tools will provide the basis of future community ecology studies of C. fumiferana baculoviral infections at both the virus species and genotypic variant level.

Poster / Viruses. V-30

The use of biolistics to infect, transfect, and co-transfect baculoviruses in larvae.

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As an alternative procedure to develop recombinant baculoviruses, we were able to infect, transfect and co-transfect T. ni larvae with the baculoviruses AcMNPV and TnGV by bombardment with microprojectiles coated with virions, viral DNA, and viral DNA and a transfer vector, respectively. A series of shooting conditions were tested until positive results were obtained. The use of 1.6 μm gold particles at 900 psi shooting pressure, 400 torr vacuum, 7 cm distance to target, on sets of 20 first-instar larvae held in a 16 mm diameter container, proved to be the best shooting conditions. Typical infection symptoms were shown by larvae when shot with viruses or viral DNA from AcMNPV or TnGV. Co-transfected recombinant AcMNPV and TnGV were identified by the formation of occlusion bodies and GFP, respectively, in bombarded larvae. This technique opens a wide range of possibilities, not only to use an extensive number of baculoviruses as expression vectors for heterologous proteins, but also be used to infect, transfect or co-transfect a wide variety of viruses into animal cells.

Poster / Viruses. V-31

Post-transcriptional processing of baculovirus late mRNAs

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Baculoviruses encode an RNA polymerase that transcribes viral late genes. This 4 subunit complex has RNA synthesis, promoter recognition, mRNA capping, and polyadenylation activities. The capping activity maps to the LEF4 subunit, which two separable domains for guanyltransferase and RNA triphosphatase activities. Construction of mutant viruses containing specific substitutions within the LEF-4 subunit showed that guanyltransferase activity was essential for viral replication while the RNA triphosphatase activity was dispensable.

The lack of a requirement for RTPase function was unexpected because this enzyme activity is required for the formation of an authentic mRNA cap structure. Therefore, we considered the possibility that the viral protein PTP, which also has RNA triphosphatase activity, might substitute for this function of LEF-4. To test this idea,

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a mutant virus that lacked both RNA triphosphatase activities was constructed. Infection studies revealed that the double-mutant virus was viable and normal with respect to the production of budded virus. Pulse-labeling studies and immunoblot analysis revealed a moderate defect in the synthesis of polyhedrin, although late gene expression was equivalent to wildtype in all other regards. To test whether capping of viral RNAs was altered in the double mutant, insect cells were infected in the presence of radiolabeled phosphate. Total mRNA was purified during the late stage of infection and the cap structure was analyzed by thin layer chromatography.
Together these results show that baculoviruses, although they encode two different types of RNA triphosphatases, replicate and express their late genes at normal levels in the absence of these proteins.

Poster / Viruses. V-32

Evidence supporting the presence of viral fibroblast growth factor on the surface of baculovirus virions

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The baculovirus Autographa californica M nucleopolyhedrovirus (AcMNPV) encodes a 21 kilodalton secreted protein with homology to various mammalian and insect fibroblast growth factors (FGFs). Previous in vitro work with this virally produced FGF (vFGF) has shown that it binds effectively to heparin sepharose and can stimulate motility of cells from various insect cell lines, both hallmark properties of previously characterized FGFs. In vivo work with a recombinant of AcMNPV lacking vfgf showed a significant delay in virally induced death in two permissive insect strains, Spodoptera frugiperda and Trichoplusia ni, yet the cause of this delay has not been established to date. Here, we report that vFGF is produced as early as 6 hours post infection and protein levels remain steady until 48 hours post infection. Interestingly, budded virus expressing vfgf showed consistently higher binding to heparin sepharose than budded virus lacking vFGF. This suggests that vFGF is present on the surface of the budded virion.

Poster / Viruses. V-33

Functional analysis of Helicoverpa armigera nucleopolyhedrovirus ORF2

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HA2 protein of the Helicoverpa armigera nucleocapsid nucleopolyhedrovirus (HearNPV) is a WASP-homology protein and capable of nucleating branched actin filaments in presence of Arp2/3 complex in vitro. In this study, we demonstrate the WCA domain of HA2 accelerates Arp2/3-mediated actin assembly in vitro and is indispensable to the function of HA2. To determine the role of ha2, the ha2 knockout and ha2 repair bacmids were constructed. Transfection and infection analysis demonstrated that the ha2 null bacmid was unable to produce infectious budded virus while the repair bacmid rescued the defect. We further repaired the ha2 null recombinant with a series of truncated ha2, and found only the recombinants containing truncated ha2 which covered the WCA domain could yield infectious virions. In addition, deletion of the C terminal of ha2, a PtdIns 4-kinase homology, dramatically decreased the viral titer. Subcellular localization analysis showed that the native HA2 and the fragments, which could rescue ha2 deletion recombinant, were distributed in the cytoplasm of the Hz-AM1 cells, and transported to the nucleus after infected with HearNPV. On the contrary, the ha2 fragments, which could not rescue ha2 deletion recombinant, were localized in the whole cell with or without infection of HearNPV.

Poster / Viruses. V-34

Ac18 is not essential for propagation of Autographa californica multiple nucleopolyhedrovirus

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Orf18 (ac18) of Autographa californica multiple nucleopolyhedrovirus (AcMNPV) is a highly conserved gene in lepidopteran baculovirus genomes and its function is unknown. In this study, an ac18 knockout bacmid containing the AcMNPV genome and an ac18 repair bacmid were generated to determine the role of ac18 in baculovirus life cycle. After transfection of Sf-9 cells, either the ac18 knockout bacmid or the repair bacmid was able to produce infectious viruses and both viruses showed similar infection pattern. Compared with wild-type AcMNPV bacmid, the deletion mutant did not reduce its infectivity for Trichoplusia ni in LD50 bioassay. Electron microscopic analysis showed the normal nucleocapsids presented in ac18 knockout virus-infected cells. In Ac18-GFP fusion virus-infected cells, fluorescence was first detected in the cytoplasm along the periphery of the nucleus and subsequently localized to intranuclear ring zone. These results demonstrate that ac18 is not essential for viral propagation in the AcMNPV life cycle.

Poster / Viruses. V-35

Mutagenesis and functional analysis of the fusion peptide of HearNPV F protein

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The fusion peptides of fusion (F) proteins of Group II NPVs are highly conserved and hydrophobic, which contain an amphiphilic helix with typical hydrophobic side and hydrophilic side. Mutageneses of the fusion peptide of Heliocoverpa armigera single nucleocapsid nucleopolyhedrovirus (HearNPV) F protein were conducted to study the functional and structural relationship of F protein. Mutant F genes with single amino acid substitutions were constructed by site-directed mutagenesis and introduced into f-null HearNPV bacmid, which were then used to transfect and infected HzAM1 cells. Results showed that two mutant Fs that of FN174G and FG176L could rescue the infectivity of f-null HearNPV, whereas three other mutant Fs that of FN174L, FI175N and FD184L could not rescue the infectivity of f-null HearNPV. Syncytium formation assay was performed to characterize the fusogenic activities of the recombinant rHaFN174G and rHaFG176L. Results showed that rHaFN174G could induce formation of larger multinucleate syncytia than that of rHaFG176L and wild-type virus. The fusogenic activity of rHaFN174G was about four-fold higher than wild-type virus, whereas the fusogenic activity of rHaFG176L was about 38% of wild-type virus. One-step growth curve of rFN174G and rHaFG176L indicated that these two mutations did not significantly affect the BV production in comparison to that of the wild-type viruses.

Poster / Viruses. V-36

Identifying the key amino acids required for nuclear localization of AcMNPV late expression factor 3 (LEF-3)

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AcMNPV is the best-studied member of the Baculoviridae family

and most of the genes identified in this virus serve as a basis for comparison to other baculoviruses. A single-stranded DNA binding protein, LEF-3 (407 aa, 45 kDa), is essential for AcMNPV DNA replication. LEF-3 also transports P143, a helicase, to the nucleus. We predict that LEF-3 has functional domains including ones responsible for ssDNA binding, P143 interaction, and nuclear localization. Site directed mutagenesis revealed that N terminal amino acids 5 to 56 are responsible for LEF-3 nuclear localization (characteristics similar to classic NLSs are found here), while amino acids 2 to 125 are required for interaction with P143. To identify amino acids within the 5 to 56 region essential for nuclear transport, conserved amino acids were targeted for mutagenesis. To identify regions possibly interacting with P143, amino acids within the first 125 residues of LEF-3 were targeted. The intracellular localization of mutated proteins was examined using fluorescence microscopy. The results of mutating conserved amino acids or deleting various regions within the N-terminal region of LEF-3 will be presented.

Poster / Viruses. V-37

Sequence analysis of the Spodoptera litura granulovirus genome

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The nucleotide sequence of the Spodoptera litura granulovirus (SlGV) genome was determined and analysed. It was 124,121 bp long, with a 61.2% A+T content and contained 136 putative open reading frames (ORFs) of 150 nucleotides or larger. The 136 putative ORFs covered 87.4% of the genome. Among these, 28 ORFs were conserved in most completely sequenced baculovirus genomes, 51 were granuloviruses (GVs)-specific, 4 were nucleopolyhedroviruses (NPVs)-specific, and 53 were present in some NPVs and/or GVs. Especially, there were 16 SlGV-specific ORFs in 51 GV-specific ORFs. Chitinase and cathepsin genes involved in the liquefaction of the infected host were not found in the SlGV genome, which explains why SlGV-infected insects do not degrade in a typical manner. When the phylogenic relationship was analyzed using the nucleotide sequence of granulin gene, SlGV was most closely related to Trichoplusia ni granulovirus (TnGV) and Xestia c-nigrum granulovirus (XcGV) which were belonged to Type I granulovirus.

Poster / Viruses. V-38

Complete sequence and organization of Antheraea pernyi nucleopolyhedrovirus, a dr-rich baculovirus

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The completion and reporting of baculovirus genomes is extremely important as it advances our understanding of gene function and evolution. Due to the large number of viral genomes now sequenced it is very important that authors present significantly detailed analyses to advance the understanding of the viral genomes. The genome of AnpeNPV, which infects Chinese tussah silkworm (Antheraea pernyi), was sequenced and analyzed. The genome was 126,629 bp in size. The G+C content of the genome, 53.4%, was higher than that of most of the sequenced baculoviruses. 147 open reading frames (ORFs) that putatively encode proteins of 50 or more amino acid residues with minimal overlap were determined. Of the 147 ORFs, 143 appeared to be homologous to other baculovirus genes, and 4 were unique to AnpeNPV. Furthermore, there are still 29 and 33 conserved genes present in all baculoviruses and all lepidopteran baculoviruses respectively. In addition, the total number of genes common to all lepidopteran NPVs is sill 74, however the 74 genes are somewhat different from the 74 genes identified before because of some new sequenced NPVs. Only 6 genes were found exclusively in all lepidopteran NPVs and 12 genes were found exclusively in all Group I NPVs. AnpeNPV encodes v-trex(Anpe115, a 3 to 5 repair exonuclease), which was observed only in CfMNPV and CfDEFNPV in Group I NPVs. This gene potentially originated by horizontal gene transfer from an ancestral host. In addition, AnpeNPV encodes two conotoxin-like gene homologues (ctls), ctl1 and ctl2, which were observed only in HycuNPV, OpMNPV and LdMNPV. Unlike other baculoviruses, only 3 typical homologous regions (hrs) were identified containing 2~9 repeats of a 30 bp-long palindromic core. However, 24 perfect or imperfect direct repeats (drs) with a high degree of AT content were found within the intergenic spacer regions that may function as non-hr, ori-like regions found in GrleGV, CpGV and AdorGV. 9 drs were also found in intragenic spacer regions of AnpeNPV. AnpeNPV belongs to Group I NPVs and is most similar to HycuNPV, EppoNPV, OpMNPV and CfMNPV based on gene content, genome arrangement, and amino acid identity. In addition, analysis of genes that flank hrs supported the argument that these regions are involved in the transfer of sequences between the virus and host.

Poster / Viruses. V-39

Comparative study of the sequence of Choristoneura biennis entomopoxvirus

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The two-year cycle budworm, Choristoneura biennis, is a major defoliator of white spruce and subalpine fir in Western Canada. Repeated defoliation causes tree mortality and loss of timber. An entomopoxvirus isolated from this insect (CBPV) has been previously characterized and its genome partially sequenced. Like other entomopoxviruses, its genome is composed of approximately 80% A+T residues. While all poxviruses appear to share common core genes, analysis of the CbEPV genome has revealed that it contained genes whose products are required for host penetration and initiation of infection. Both EPVs and chordopoxviruses must, therefore, have similar strategies to enter the host. Entomopoxvirus, however, do not have a homologue to CrmA gene, which is related to inhibition the apoptosis in cells infected with chordopoxvirus. Therefore, it appears the EPVs and the chordopoxviruses have acquired different anti-apoptotic genes. EPVs have a homologue to the baculovirus antiapoptotic gene p35. Apart of iap genes, two other anti-apoptotic genes were found in the CBPV genome, both bearing structural similarities to p35 and p49. Phylogenetic studies indicate that the two CBPV genes might have been acquired from two different sources.

Poster / Viruses. V-40

Role of the baculovirus P143/LEF-3 complex in viral DNA replication

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AcMNPV has a broad host range while CfMNPV is very host specific, infecting only Choristoneura fumiferana (spruce budworm). Our previous results suggested that the P143-LEF-3 complex is a host specificity factor for baculovirus replication. To test this hypothesis, a series of gene-specific knockouts in AcMNPV bacmids were generated. AcMNPV lef-3-, p14-3- or a double lef-3-/p143-knockout were generated by homologous recombination in E. coli. A series of rescue bacmids were then constructed which expressed the homologous AcMNPV genes, or the heterologous CfMNPV genes. Sf-21 cells transfected with the various bacmid DNAs were examined for early and late protein expression, and polyhedra production. Supernatants of the bacmid-transfected cells were titrated for the presence of budded virus. Viral DNA replication was studied by real time PCR. The results confirm that AcMNPV lef3 and p143 are essential for DNA replication in vivo. AcMNPV replication in Sf21 cells was rescued by the replacement with both CfMNPV lef-3 and p143 homologues but the level of replication was much lower than with the AcMNPV bacmid. Polyhedra were observed in transfected cells, but the titre of budded virus was lower than AcMNPV. These results suggest that baculovirus replisome assembly and function requires very specific protein-protein interactions that may be virus species specific.

Poster / Viruses. V-41

The 5' untranslated region of Perina nuda virus (PnV) possesses a strong internal translation activity in baculovirus infected insect cells

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A bi-cistronic baculovirus expression vector and fluorescent protein-based assays were used to identify the sequences that possess internal translation activity in baculovirus infected insect cells. We demonstrated that the 5'UTR (473 nucleotides) of Perina nuda virus (PnV) and 5'UTR (579 nucleotides) of Rhopalosiphum padi virus (RhPV) but not the IRES sequence of Cricket paralysis virus have an internal translation activity in baculovirus infected Sf21 cells. In addition, we found that including the first 22 codons of the predicted PnV ORF (for a total of 539 nucleotides) enhanced the internal translation activity by about 18 times. This is the first report of an internal translation activity for baculovirus expression system (BEVS) in the iflavirus 5' sequence and may facilitate the development of polycistronic baculovirus transfer vectors that can be used in BEVS for the production of multiple protein complexes.

Poster / Viruses. V-42

Restricted gene transcription of Autographa californica multiple nucleopolyhedrovirus (AcMNPV) in mammalian cells

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Autographa californica multiple nucleopolyhedrovirus (AcMNPV) is the type species of the Baculoviridae. NPV infects several important insect pests and is used for biopesticide. AcMNPV can also enter the mammalian cells so that used as the gene delivery vector. Our works focused on the behavior of AcMNPV in mammalian cells, especially their transcription activity. We found that AcMNPV could express several their genes in human HeLa cells and mouse BHK cells though these transcription level is very low. Although these expressed genes are mainly categorized in early genes that have the CAGT motif in their promoter recognized by cellular RNA polymerase II, some late genes transcripts were also detected. Almost all early genes expressed were transcribed from CAGT motif in their promoter except for pe38. However, late genes lacking CAGT motif in their promoter sequence were transcribed from incorrect initiation sites in the coding regions. These results clearly showed that the AcMNPV genome acts as a template for transcription in mammalian cells through the usual infection pathway and that one of the key steps which prevents AcMNPV from replicating in mammalian cells is transcriptional regulation. We here discuss the restricted transcription from AcMNPV genome in mammalian cells in more detail.

Poster / Viruses. V-43

Determination of the promoter region of the Chilo iridescent virus DNA polymerase gene

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Chilo iridescent virus (CIV) is a member of the family Iridoviridae and occurs in insects. The DNA genome (212,482 base pairs) is entirely sequenced, but very little is known about viral gene regulation, expression and function. We investigated the transcriptional regulation of the CIV exonuclease gene (012L). Infection of Bombyx mori SPC-BM-36 cells in the presence of Ara-C (inhibits DNA replication) or cycloheximide (inhibits protein synthesis), followed by RT-PCR on isolated total RNA, showed that exonuclease is expressed as an immediate-early gene. The temporal expression of the gene is also examined. Detecting the RNA transcript of the exonuclease early at infection confirmed the data about the class of the gene obtained with the inhibitors. 5'RACE analysis on RNA isolated from CIV-infected Bm cells showed that transcription initiation site is located 30 nucleotide upstream of the translational start codon. To determine the limits of the putative promoter, up¬stream sequences of various lengths were cloned in front of a firefly luciferase reporter gene. The resulting plasmid constructs were tested in a transfection assay, in which the baculovirus IE-l promoter fused to Renilla luciferase was used as an internal control for transfection efficiency. Exonuclease promoter was only active when cells were simultaneously infected with CIV. A gradual reduction in luciferase expression occurred as the deletions extended from –200 to –10, relative to the mRNA start site. The AAAAT motif responsible for the key element of promoter activity for CIV DNApol gene is also included at the 100 nt upstream of the translational start codon of exonuclease gene.

Poster / Viruses. V-44

Annotation and expression profiling of presumptive apoptosis regulatory genes in the yellow fever mosquito, Aedes aegypti

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Apoptosis has been extensively studied in Drosophila both by biochemical and genetic approaches, but there is a lack of knowledge about the molecular mechanisms of apoptosis regulation in other insects. In mosquitoes, apoptosis has been shown to occur during malaria infection in the midgut of anopheline species and during arbovirus infection in midgut and salivary glands in culicine and aedine species, suggesting that apoptosis may play a role in mosquito innate immunity. Using the available genome sequence of Aedes aegypti, we mined for presumptive apoptotic regulators by

performing BLAST searches and overlapping EST analysis, using well-characterized Drosophila proteins and predicted proteins from Anopheles gambiae as querries. Using this bioinformatics approach we found homologs corresponding to ten caspases, three inhibitor of apoptosis (IAP) proteins, and four other proteins that have been shown to regulate caspases in Drosophila. We analyzed expression of these genes by real-time RT-PCR in Ae. aegypti larvae, pupae and adults. For some of the candidate genes we found restricted expression while others were expressed in all stages and tissues analyzed. This study represents a necessary first step in elucidating the molecular mechanisms of apoptosis regulation in Ae. aegypti.

Poster / Viruses. V-45

A strategy for genetic modification of Epinotia aporema Granulovirus

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Epinotia aporema (Lep. Tortricidae) is a major pest of legume crops in South America. A granulovirus (EpapGV) characterized in our laboratory exhibits a great potential as bioinsecticide; however it cannot compete with chemical insecticides when the average temperature is under 20oC. In this context, there is room for improvement of its biological performance using genetic engineering. This type of manipulation proved to be difficult to manage without a susceptible cell line. Therefore, we set out to construct a bacmid by inserting a miniF replicon into EpapGV DNA. The egt gene has been previously characterized and its deletion in other baculoviruses led to an enhancement of the speed of kill. Thus, a construct containing EpapGV egt 5' and 3' regions was generated to target the miniF sequences to this locus. The following steps required the introduction of the bacmid into E. aporema larvae. Transfection was first tested with authentic viral DNA isolated from granules. To set up the experimental protocols we used Anticarsia gemmatalis Nucleopolyhedrovirus (AgMNPV), because the availability of the susceptible cell line UFLAg-286 facilitated testing procedures. The propagation of EpapGV DNA in E. coli will simplify the generation of site-specific mutants both for basic studies and enhancement of insecticidal activity.

Poster / Viruses. V-46

Baculovirus budded virus Affinity on for Heparin Sepharose is Columns Partially Mediated by a Virally Produced Fibroblast Growth Factor

Chris Lehiy, Chanitchote Devisitssakun, and A. Lorena Passarelli

The baculovirus Autographa californica M nucleopolyhedrovirus (AcMNPV) encodes a 21 kilodaltonDa secreted protein with homology to various mammalian and insect derived fibroblast growth factors (FGFs). Previous in vitro work with this virally produced FGF (vFGF)fibroblast growth factor (vFGF) has shown that it binds effectively to heparin sepharose beads and can stimulate cell motility of cells from various insect cell lines, both hallmark propertiess of previously characterized FGFs. In vivo work with a recombinant of fgf deletion AcMNPV lacking vfgf baculovirus has shownshowed a significant delay in virally induced death in two permissive insect strains, Spodoptera frugiperda and Trichiplusia ni, yet the cause of this delay has not been established to date. Here, we report that vFGF is produced as early as 6 hours post infection and protein levels remain steady continues until 48xx hours post infectionsteadily throughout the initial budded virus production cycle. Interestingly, budded virus expressing vfgf showed produced from cells infected from either a wildtype virus or the deletion fgf virus show consistently higher binding to heparin sepharose than budded virus lacking vFGF. when vFGF in produced. This suggests leads to the possibility that vvirally encoded FGF is being carried on thepresent on the surface of the budded virion.

Poster / Viruses. V-47

Identification of virion proteins of Spodoptera frigiperda ascovirus by mass spectroscopy

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Ascoviruses (family Ascoviridae) are double-stranded DNA viruses with a circular genome, and they attack lepidopterans in which they produce large, allantoid enveloped virions, 150 nm in diameter by 400 nm in length. Although the genome of the type species, Spodoptera frugiperda ascovirus 1a (SfAV1a) was recently sequenced, comparative analysis of protein sequences with those of other invertebrate viruses revealed little regarding the identity of structural and other proteins that constitute the SfAV1a virion. Therefore, virions were fractionated by one-dimensional SDS-PAGE, isolated, and analyzed by nano-liguid chromatography/tandem mass spectrometry (nano-LC MS/MS). Twenty proteins, encoded by the SfAV1a were identified. These included a helicase (ORF009), S1/P1 nuclease (ORF075), Serine/threonine-like protein kinase (ORF064), inhibitor of apoptosis (ORF015), Thiol oxidoreductase-like protein (ORF061), CTD phosphatase (ORF109), Yabby-like transcription factor (ORF091), Cytochrome oxidase assembly factor (ORF002), major capsid protein (MCP, ORF041) and another structural protein, P64 (ORF048). To confirm the presence of the latter two proteins in the SfAV1a virion, Western blot analysis was performed using antibodies raised against recombinant MCP and P64 proteins produced using the Bac-to-Bac Baculovirus expression system. Data obtained from these studies provide a foundation for understanding how these proteins interact to form the complex structure of the SfAV1a virion, as well as for elucidating mechanisms involved in SfAV1a entry into host cells and early events of SfAV1a virogenesis.

Poster / Viruses. V-48

Sf29 is a viral factor that could be involved in virion packing within the OBs

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During plaque assay purification of the SfMNPV wild-type population, we found genotypic variants showing various levels of per os infectivity. Variants lacking the ORF homologue to Se030 (Sf29) had lower pathogenicity and virulence. To determine the effect of disrupting Sf29 in SfMNPV pathogenesis, we used a PCR and bacmid-based recombination system to delete the Sf29 gene from an SfMNPV bacmid. Different aspects of virus replication and pathogenesis were studied. The Sf29null bacmid was able to generate a transmissible infection in cell culture and S. frugiperda larvae. We therefore concluded that Sf29 is not essential for propagation of viral infection. Temporal expression revealed that Sf29 is transcribed at a similar level and just 12 h before Sfpolh in larvae. SfMNPV WT, and SfMNPV, Sf29null and Sf29rescue bacmid OBs were produced. Six times less DNA was found in polyhedra produced by the Sf29null bacmid. We therefore investigated ODV content per occlusion and nucleocapsid distribution among ODV populations.

No differences were found in nucleocapsid distribution among the ODV populations. However, the ODV banding pattern was less intensive in the Sf29null bacmid. We determined by end point dilution in Sf21 cells that Sf29null OBs produced ~8 times less ODVs. By intrahemocelic infection of the same amount of DNA, we found that Sf29null DNA was as pathogenic and virulent and produced the same amount of DNA/larva and OBs/larva as SfMNPV WT, and SfMNPV and Sf29rescue bacmid DNAs. However less ODV are packaged within the OBs. The presence of less ODV per occlusion explained the lower pathogenicity and virulence of Sf29null bacmid OBs. The Sf29 could be involved in ODV packing within the OBs during the SfMNPV replication.

Poster / Viruses. V-49

Functional and phylogenetic comparisons of viral homologues of a protein lethal to endoparasitoids

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When the gregarious endoparasitoid Cotesia kariyai parasitizes Mythimna separata larvae infected with an entomopoxvirus (MyseEPV), its embryos and larvae die inside the host. Previous studies showed that a factor lethal to C. kariyai was present in virion-free plasma (VFP) from M. separata larvae infected with MyseEPV. A 28-kDa polypeptide was purified and named PLCK (protein lethal to C. kariyai). The plck gene consists of a 1,280-bp open reading frame. Homology searches and PCR with degenerate primers revealed that plck homologues occur in eight baculoviruses. A phylogenetic tree based on the partial sequence most highly conserved region of plck (120 bp) was incompatible with baculovirus genome trees. Parasitoid-killing activities were found in Xestia c-nigrum granulovirus (XecnGV), Helicoverpa armigera GV, the Hawaiian strain of Pseudaletia unipuncta GV (PsunGV-H), and Mamestra configurata nucleopolyhedrovirus B (MacoNPV-B). Western blotting using an anti-PLCK polyclonal antibody revealed that VFP from larvae infected with PsunGV-H, XecnGV, and MacoNPV-B reacted with the antibody. Interestingly, VFP from MyseEPV-infected larvae showed parasitoid-killing activities not only to C. kariyai larvae but also to four other braconid parasitoids including Microplitis manilae and Apanteles glomeratus. These finding suggest that plck homologues have been conserved in several baculoviruses to overcome competition with braconid parasitoids.

Poster / Viruses. V-50

Determination of the occlusion-derived virus proteins of Xestia c-nigrum granulovirus

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Transmission of Baculoviruses occurs by the oral route with occlusion body, and envelope proteins of occlusion-derived virus (ODV) play important roles for establishment of infection in the host midgut. Although there is accumulation of knowledge about ODV of nucleopolyhedroviruses, only few studies have attempted for granuloviruses (GVs). Recently, the availability of genome sequence offers the opportunity to further expand our knowledge about the protein composition of ODV using a proteomic approach. Whole genome sequence analysis of the Xestia c-nigrum GV (XecnGV) has identified 181 putative genes, yet only a few of them are studied in detail. In order to identify the ODV proteins of XecnGV, especially envelope proteins, XecnGV virions were treated with Triton X-100, and soluble proteins were fractionated by SDS-PAGE and subjected to NanoLC-MS/MS analysis. Acquired and processed MS/MS data were searched against NCBI database using the Mascot search program. As a result, we confirmed that the presence of known ODV-associated proteins in XecnGV as follows: PIF-1, PIF-2, p74, ODV-E18, ODV-E25, ODV-E56, ODV-E66, 44.8kDa (ODV-EC43), vp39 (capsid), gp41, gp33, 49kDa, and Enhancin-1, -2, -3 and -4. In addition, 9 open reading frames were reported for the first time as being expressed.

THURSDAY, AUGUST 16TH

CONTRIBUTED PAPERS, Thursday, 8:00 - 10:00

FUNGI 3

Contributed Paper. Thursday, 8:00. (156)

Evaluation of whey as a basal media ingredient for mass production of Beauveria bassiana and Metarhizium anisopliae

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Spore production of Beauveria bassiana and Metarhizium anisopliae was studied in novel whey-based culture media. Spore yield and viability was determined for two B. bassiana (GHA-726 and CA-603) and two M. anisopliae (CA-1 and IMI 330189) isolates following production in three whey-based systems: solid, liquid and a diaphasic production system. Our study indicated that whey powder can effectively be used for production of spores of entomopathogenic fungi. However, spore yield and viability were significantly influenced by fungal isolate, whey concentration and the type of production process used. Under the conditions defined in the present study, spore yield ranging from 1.3 x 109 – 10 x 1011 spores l -1 of whey medium could be obtained depending on the strain and production process used. Our study revealed that spores produced by all strains in whey-based solid and liquid media showed between 73 - 99 % viability; germination rates were comparable to those obtained using the standard SDA medium. In the two-stage production process, conidia produced by GHA-726 and CA-603 were 32 – 98 % viable; viability was correlated with whey concentration. Germination of conidia produced by isolate CA-1 ranged from 66 – 93 %, and was lower than for either GHA-726 or CA-603. We believe that spore yield and viability could be improved by careful selection of whey content in the medium, incorporation of critical additives and optimization of culture conditions.

Contributed Paper. Thursday, 8:15. (157)

Effect of culture medium on Paecilomyces fumosoroseus morphogenesis, growth and production and properties of infective propagules

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Paecilomyces fumosoroseus, one of the most infective fungal species for the whitefly Bemisia sp., produces infective propagules (IP) in submerged fermentation. In order to optimize a culture medium and to know the role of some components, a Hadamard matrix and a basal medium containing glucose, NH4NO3, and minerals in a Na2EDTA solution at pH 5.5 were used. Indeed, growth and production and properties of IP were affected by cations. Mo(VI) and Fe (II) had a slightly positive effect on mycelia and IP production, but Zn(II) had the highest. Usually, IP are white; however, the addi-

tion of Cu(II), and mainly Fe (II), conferred them the characteristic pale brown color of aerial conidia. The color is attributed to melanin formation, which is involved in UV light resistance. Besides, IP produced in the optimized media resisted better high temperatures (LT50, 43oC) than those produced in Jackson's media, (LT50, 40oC). At high glucose and NH4NO3 concentrations (50 g/L and 1.84 g/L respectively, aw approx. 0.993), free mycelia and abundant IP (1.6-4.0×108 propagules/mL) were formed, while at low concentrations (10 g/L and 0.5 g/L repectively, aw approx. 0.998), the fungus formed pellets and IP production decreased (4-160×104 propagules/mL).

Contributed Paper. Thursday, 8:30. (158)

Further research on the production, longevity and infectivity of the zoospores of Leptolegnia chapmanii Seymour (Oomycota: Peronosporomycetes)

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The effect of temperature on the production, survival and infectivity of zoospores of an Argentinean isolate of Leptolegnia chapmanii was determined under laboratory conditions. Zoospore production by L. chapmanii in vitro and in vivo upon 1st and 4th instar larvae of the mosquito Aedes aegypti was studied at three different temperatures. Zoospores from infected larvae were infective to mosquito larvae for 5, 51, and 12 consecutive days when incubated at 10, 25, and 35oC, respectively. Maximum zoospore production in infected 4th instar larvae was 9.6 ± 1.4 x 104 zoosp/larva (61,000 encysted and 35,000 swimming zoospores) after 48 h at 25oC, The average production of encysted + flagellated zoospores by individual infected 4th instar Ae. aegypti larvae was 3.57±0.46 x 105 zoospores for 6 consecutive days at 25oC. Zoospore production in vitro was also affected by temperature with a maximum of zoospores (n=47,666/ml) produced at 25oC. When zoospores produced in vitro were used as inoculum against Ae. aegypti larvae at 25oC, larvae mortality was recorded for 5 consecutive weeks. Temperature directly affected infectivity and production of zoospores in vivo and in vitro.

Contributed Paper. Thursday, 8:45. (159)

Development of a formulation of Beauveria on nonwoven fabric strips for control of Monochamus alternatus (Coleoptera: Cerambycidae)

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Registration of a formulation of Beauveria bassiana cultured on nonwoven fabric strips was approved as a commercial mycoinsecticide in February 2007 in Japan. This formulation targets Monochamus alternatus to prevent the pine wilt disease causing serious damages onto pine forests in Japan and other East Asian countries. Evaluation of this formulation had been carried out in various areas of Japan. When applied this onto the bark of nematode-infested pine trunks in summer, larvae of M. alternatus inhabiting under the bark were killed at sufficiently high mortalities. Adults of M. alternatus are less susceptible to this fungus, but direct contact with B. bassiana conidia on the formulation could produce high mortality of the adults within a short period. This method cannot be used on healthy trees for prevention application, but can be used to kill the adults emerging from the nematode-infested trees. The adults infected with B. bassiana reduced the amount of maturation feeding, and thus, transmission of the pinewood nematode to healthy pine twigs was almost completely inhibited. This type of formulation is efficient to retain an entomopathogenic fungus on tree bark, and it is thought to be a good control against other wood boring insects, too.

Contributed Paper. Thursday, 9:00. (160)

Investigating the structure of natural populations of Beauveria bassiana occurring in different habitats

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Poor understanding of the population biology of anamorphic Ascomycete entomopathogenic fungi may well be acting as a barrier to the development of new and more effective ways of exploiting them for biological control. It also impacts on bio-prospecting and risk assessment studies for fungal biocontrol agents. We have been investigating the structure of natural Beauveria bassiana populations sampled in England, inspired by the insights of Bidochka and others on the relationship between habitat type and fungal population structure. Isolates of B. bassiana (and other entomopathogenic fungal species) were obtained from c. 4000 soil samples collected from woodlands or grass pastures in a broad transect from south-west to mid-west England. Frequency of occurrence of B. bassiana and other species increased with more northerly latitudes. Multi locus sequence data were obtained for a total of 120 B. bassiana isolates from each sample location. The amount of recombination within clades, evidenced from network analysis, differed markedly depending on the habitat association of the clade. In a second experiment, the same structural pattern was observed for isolates sampled from a complex of fields and hedgerows on the University farm, with hedgerow acting as a substitute for woodland.

Contributed Paper. Thursday, 9:15. (161)

Association of Beauveria spp. with Bark Beetle Populations in Pinus radiata Plantations in New Zealand

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First recorded in New Zealand in 1929, the pine bark beetle, Hylastes ater, is now established in all exotic pine plantations. Year-round harvesting of Pinus radiata ensures a continual supply of breeding habitat (stumps and similar logging waste), allowing populations to build to epidemic levels. Maturation feeding by emerging adults around the root collar of seedlings planted alongside harvested areas can significantly impact regenerative plantings. H. ater also vectors sapstain and other damaging fungi, and can transmit these to live seedlings during sub-lethal feeding attempts.

Beauveria caledonica is the predominant naturally occurring pathogen of H. ater and Hylurgus ligniperda in New Zealand; laboratory bioassays of these and other strains have confirmed their potential for biocontrol. Before field testing, it is important to survey a range of forest sites to determine the level and variation of fungi present in the beetles' environment. This information is valuable in the selection of the best strains (i.e., associated or non-associated strains) for control, suitable targets, formulation and delivery systems, and in defining application rates. This paper reports on Beauveria spp. recovered from different substrates associated with H. ater (soil, bark, cambium/frass from the feeding zone, and beetles), their relative pathogenicity, and molecular characterisation.

Contributed Paper. Thursday, 9:30. (162)

Molecular characterization and comparative virulence of Beauveria bassiana isolates associated with the shore fly, Scatella tenuicosta, in greenhouses

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John D. Vandenberg2 , and Stephen P. Wraight2
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Reports of natural epizootics of the insect pathogenic fungus Beauveria bassiana in greenhouse and laboratory populations of Scatella tenuicosta, a nuisance pest and vector of plant pathogens, suggest its potential for microbial control of this pest. In this study we assessed the genetic diversity of B. bassiana isolates found associated with a colony of shore flies established from a lettuce production facility in NY, and compared these isolates to two commercial B. bassiana strains. Microsatellite markers were used to assess genetic variation among isolates collected from adult and pupal S. tenuicosta, adult Hexacola neoscatellae, a hymenopteran parasitoid of shore flies, and algae, the food source of immature and adult shore flies. Twenty-five single spore isolates were resolved into three distinct genotypes using 10 microsatellite markers. Two genotypes were common and were similar to indigenous strains in agricultural fields in the northeastern U.S., suggesting that these genotypes are not specific to shore flies. The third genotype, however, was observed in only one isolate from a shore fly pupa. We also developed a bioassay and compared the virulence of representative isolates of the three genotypes and commercial strains GHA (BotaniGard) and ATCC74040 (Naturalist) against third instar, pupal and adult shore flies.

Contributed Paper. Thursday, 9:45. (163)

Horizontal transmission possibility of the fungus Beauveria bassiana KCF102 by mating behavior between Sunn pest, Eurygaster integriceps (Hem., Scutelleridae) adults

Reza Talaei-Hassanloui and Aziz Kharazi-Pakdel
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The cosmopolitan entomopathogenic fungus, Beauveria bassiana (Balsamo) Vuillemin (Ascomycota, Hypocreales) is an effective biological control agent for reducing densities of insects from most orders. Wind currents, rain splash from soil surfaces and insect activities could potentially contribute to the distribution of this fungus inoculum. We sought to investigate the possibility of horizontal transmission of B. bassiana by mating behavior between Sunn pest, Eurygaster integriceps (Hem., Scutelleridae) adults by allowing inoculated adults to mate with non-inoculated ones. Since it is important to know which parts of body is more susceptible than the others and whether these parts of males and females contribute or contact with each other in mating behavior, susceptibility to B. bassiana of different parts; antennae, pronotum, tarsi, ventral abdomen and total body was examined. There was no significant difference for susceptibility to B. bassiana of male and female adults. There were significant differences among treatments for mortality of non-inoculated adults that meant horizontal transmission could be happened between inoculated and non-inoculated adults but being no significant difference between two defined treatments of this experiment revealed that this kind of horizontal transmission was not due to the mating behaviour as the same transmission could be existed between inoculated and non-inoculated males or between those of female adults. There was a significant difference for mortality of adults among the five body treatments, ranged between 16.6 and 48.9 % with the highest mortality for total body treatment and the lowest one for the pronotum treatment.

CROSS-DIVISIONAL SYMPOSIUM, Thursday 8:00 - 10:00

Battling Alien Invaders: Development and use of Entomopathogens to Control Invasive Insect Pests.

Symposium. Thursday, 8:00. (164)

Development and production of the Lymantria dispar nucleopolyhedrovirus as a microbial control agent for the gypsy moth

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The Forest Service initiated a research and development programs in the late 1950s to develop the Lymantria dispar nucleopolyhedrovirus (LdNPV) as a microbial control agent for the gypsy moth. This effort included many partners in other US government agencies, academia, and industry, and culminated in 1978 when the virus was registered by the U.S. Environmental Protection Agency for gypsy moth control under the name Gypchek. LdNPV is produced by a joint Forest Service and Animal and Plant Health Inspection Service effort in gypsy moth larvae. Sufficient virus to treat from 5000 – 7000 acres is produced each year, and is used in areas inhabited by species that are threatened by the use of broad-spectrum pesticides. Annual use of LdNPV has varied from about 3000 – 18,000 acre equivalents. More recently, research efforts have focused on development of virus strains that can be produced in cell culture and methods for virus production in cell culture bioreactors. All currently available gypsy moth cell lines have been assessed for in vitro production, and a line derived from the Ld652Y cell line exhibits the best overall production characteristics. Virus has been successfully produced in both stirred tank and disposable bag bioreactor systems, with the bag system giving higher yields of virus polyhedra.

Symposium. Thursday, 8:20. (165)

Releasing exotic microsporidia into North American gypsy moth populations: regulations and other issues

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Release of exotic pathogens into invasive host populations as classical biological control agents requires a reliable understanding of host and pathogen biology and interactions, taxonomic placement, and environmental safety. Several species of microsporidia, obligate protistan pathogens related to Fungi, are commonly recovered from European gypsy moth, Lymantria dispar, populations in Europe but have never been found in North American populations. We plan to release three of the European species, Nosema portugal, Nosema lymantriae, and Vairimorpha disparis, in the U.S. in the spring of 2008. European and U.S. scientists cooperated to conduct a suite of studies that provided the data necessary to produce a North American Plant Protection Organization (NAPPO) proposal to release these microsporidia. The proposal received a favorable ruling from EPA and USDA APHIS, and the State of Illinois. An overview of the experimental work completed and the procedures necessary for filing a NAPPO proposal will be discussed.

Symposium. Thursday, 8:40. (166)

Entomophaga maimaiga and the Gypsy Moth in North America: Toward Predicting Epizootics

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The gypsy moth was introduced from France to the Boston area in 1868 and quickly became a serious defoliator of northeastern forests as it spread slowly to the south and west. Entomophaga maimaiga was purposefully introduced from Japan to the Boston area in 1910-

1911 to control outbreak populations of gypsy moth, Lymantria dispar. By 1912, E. maimaiga was reported as not established and this fungus was not reported again in North America until 1989, after which time it spread across the gypsy moth distribution. Results from molecular studies suggest that the fungus found in 1989 and after in North America is probably a more recent accidental introduction. Extensive non-target studies have demonstrated that this fungus is highly host specific in the field. Use of this fungus for augmentation has been limited due to lack of mass production although we can now produce resting spores in vitro and can avoid resting spore dormancy. This fungus will persist, spread and increase in gypsy moth populations on its own, given amenable conditions. We are presently working on the ability to predict epizootics, which would be helpful to land managers who could then avoid ground and aerial applications of chemical pesticides.

Symposium. Thursday, 9:00. (167)

Potential uses of Beauveria bassiana GHA for management of the emerald ash borer, Agrilus planipennis Fairmaire (Coleoptera: Buprestidae)

Leah S. Bauer1, and Houping Liu12
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In 2002, emerald ash borer (EAB), a woodborer native to northeastern Asia, was identified as the cause of ash tree (Fraxinus spp.) mortality in Michigan and Ontario. EAB is now known to have spread to Ohio, Indiana, Illinois, Maryland, and Virginia through the inadvertent transport of infested ash nursery stock, logs, firewood, and natural dispersal. We began studying the potential use of entomopathogenic fungi for management of EAB due to their prevalence as natural enemies in Michigan field populations. After comparing the virulence of fungal species and strains, we found B. bassiana GHA most virulent. Initially, we studied this strain, formulated as BotaniGard® ES, in laboratory, greenhouse, and small field trials to determine the best methods, timing, rate, etc. In 2004, we evaluated the efficacy of B. bassiana GHA applications, sprayed to drip four times during the summer, on trunks and crowns of 20-year-old green ash trees infested with EAB. That fall, we found 46% fewer EAB larvae in sprayed vs. control trees. The following spring, the sprayed trees showed 42% less crown dieback and had 63% fewer emergent EAB adults. In collaboration with scientists at USDA ARS, we initiated an expanded field trial in Michigan during 2006.

Symposium. Thursday, 9:20. (168)

Thelohania solenopsae as a factor of fire ant populations

David Oi
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The inadvertent introduction of fire ants into the United States over 70 years ago initially resulted in large-scale efforts to eradicate the invasive pest. Large populations, mobility, and ability to occupy diverse habitats make fire ants a dominant arthropod in infested regions and very difficult to eradicate. Current control strategies in the U.S. now focus on reducing populations to tolerable levels at specific sites, typically with insecticides. The fire ant pathogen Thelohania solenopsae was discovered in the 1970's in South America, but not until the 1990's were reductions in the field formally documented with this pathogen. Introductions of T. solenopsae into red imported fire ant populations in the U.S. spread and are self-sustaining, primarily in the fire ant social form that contains several queens per colony. T. solenopsae infected sites had population reduction of over 60% and infections in colony founding queens. Perhaps the most compelling effect of T. solenopsae is the delay in re-infestation in areas cleared of fire ants by insecticides. The use of T. solenopsae and other biological control agents provide a potential opportunity for the regional suppression or at least impediment to the geographic expansion of fire ants.

Symposium. Thursday, 9:40. (169)

Tritrophic interaction in the control of Sirex woodwasp by nematodes

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The mutual reliance of the woodwasp, Sirex noctilio, and the nematode, Beddingia siricidicola, on the symbiotic fungus of the woodwasp is a key element in the successful control by the nematode of this highly invasive pest of Pinus. S. noctilio was initially identified as a significant threat to Pinus plantations in Australasia and has since emerged as a significant pest in South America, South Africa, and North America. B. siricidicola was initially employed in Australia during the 1970s in an augmented biological control program with great success. However, the success of this control measure when S. noctilio is in an invasive phase is highly dependent on a thorough appreciation of the biology of the pest, its symbiotic fungus and the nematode, especially as each interacts with the tree. This presentation will address recent discoveries that are crucial to the continuing success of control programs as S. noctilio expands its range as a pest.

CONTRIBUTED PAPERS, Thursday 8:00 - 10:00

MICROBIAL CONTROL 3

Contributed Paper. Thursday, 8:00. (170)

Use and formulation of Baculovirus insecticides in Australian broadacre crops.

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Nucleopolyhedrovirus (NPVs) insecticides are established tools in pest control and insecticide resistance management in Australian cotton and grains. Research and development by the Queensland Department of Primary Industries has led to the manufacture and registration of three commercial NPV insecticides against Helicoverpa armigera, a serious pest of crops in Australia.

A range of commercial additives and reducing sugars, including mannitol, were tested for improvement in NPV efficacy in cotton. While many additives enhanced performance to some degree, there was a significant increase in efficacy with increased relative sweetness. Mannitol was found to have an antifeedant effect and lower relative enhancement of activity compared to a significant increase in efficacy with high sweetness additives.

Increased sugar concentration and combination with emulsified spray oils significantly increased NPV efficacy, as did ULV application of NPV in oil compared to conventional application in water. Results demonstrate the efficacy of practical, low-cost additives to enhance NPV field efficacy.

The successful adoption of biopesticides in Australian broadacre cropping provides a model system for the introduction of biopesticides in conventional farming systems and has resulted in industry demand for biopesticides for sucking pest management in genetically modified crops.

Contributed Paper. Thursday, 8:15. (170,1)

Suppressing plum curculio (Coleoptera : Curculionidae) with biopesticides

Renee Pereault, Mark Whalon, Department of Entomology, B11 Center for Integrated Plant Systems, Michigan State University, East Lansing, MI 48824

Eastern US tree fruit growers, facing limitations on pesticide inputs largely brought on by the passage of the Food Quality Protection

Act (1996), are challenged by the lack of efficacious tools to manage internal fruit feeding plum curculio larvae (Conotrachelus nenuphar Herbst). An overview of the pest status and life history of plum curculio in relation to current management techniques is presented with a focus on the potential of augmentative application of entomopathogenic fungi and entomopathogenic nematodes to manage both adult and larval life stages. Results of tree fruit field trials focusing on the commercial spray formulation and a granular formulation of Beauveria bassiana strain GHA applied against plum curculio larvae will be presented. These field trials were supported by an array of laboratory bioassay results obtained by exposing plum curculio larvae to commercial Beauveria bassiana strain GHA, Metarhizium anisopliae strain F52, Steinernema carpocapsae, Steinernema feltiae and Steinernema riobrave.

Contributed Paper. Thursday, 8:30. (170,2)

Identification of the midgut receptor for Cry4Ba toxin in Anopheles albimanus larvae.

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The occurrence of arthropod borne human diseases continues to pose significant health risks in many parts of the world. The ability to control insect vectors for these diseases is compounded by the resistance of some vector populations not only to chemical insecticides, but more recently also to Bacillus sphaericus toxins. An important alternative for control of these insect vectors is through the use of Cry toxins present in Bacillus thuringiensis subsp. israelensis (Bti), which are toxic to different mosquito species.

Anopheles albimanus is the principal vector for the transmission of malaria in México. Although several anopheline species are poorly controlled by Bti, A. albimanus is an exception to this rule. Little is known about the receptor molecules that are the targets of Cry mosquitocidal proteins. We have identified by ligand-blot experiments a 60 KDa protein that binds toxin Cry4B. This protein is anchored by GPI to the membrane, as other Cry receptors like aminopeptidase N and alkaline phosphatase. The identity of this protein remains to be solved. Once identified, we propose to silence the expression of this protein in A. albimanus by RNA interference to prove their functionality as a receptor of Cry4B toxin.

Contributed Paper. Thursday, 8:45. (172)

Bioactivities of Photorhabdus luminescens subsp. akhurstii, a symbiont of entomopathogenic nematode, Heterorhabditis brevicaudis

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An entomopathogenic nematode, Heterorhabditis brevicaudis TG01, was isolated from sampled soils for the first time in Taiwan using the Galleria-bait method. Identification of the nematode was mainly based on observations under scanning electron microscopy (SEM) and nucleotide sequence of the internal transcribed spacer 1 (ITS1). P. luminescens subsp. akhurstii was isolated from nematodes and identified by phenotypic, biochemical tests, 16S rRNA and Biolog identification system. In this study, supernatant fluid of the bacterial culture was centrifuged, filtered, and bioassayed against 5 key pests of vegetables. It exhibited insecticidal activity against Plutella xylostella. Antagonistic effects of bacterial culture and protein preparations on 18 species of fungi and 12 species of bacteria were examined by means of dual or concomitant culture methods. High antimicrobial activities against Glomerella cingulata, Colletotrichum musae, Xanthomonas spp. and Erwinia spp. were observed. Studies were also carried out to test the preventability of mango anthracnose during storage with local P. luminescens and showed satisfactory results. This report also showed that the secondary metabolites from culture filtrate of P. luminescens had nematicidal properties. Efforts were also concentrated on cloning and expression of insecticidal toxin genes and lipase gene, setting up the purification methods for insecticidal toxin proteins from P. luminescens with preliminary results.

Contributed Paper. Thursday, 9:00. (173)

Novel Controlled-Delivery Formulation Technologu: Mosquito Biolarvicide Applications

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Controlled-delivery formulation technology (Matricap®) based on the use of patented coating complex admixtures composed of one or more fatty acids, fatty alcohols, fatty alcohol esters, fatty acid esters, phthalyl esters, waxes, or plasticizers, with or without one or more polymer binders and formulation aids was developed to regulate the release rate and release profile of bioactive agents form solid carrier matrices. Selection of a coating or coating complex was dependent on the type of aquatic habitat, the physicochemical characteristics of the bioactive agent, and the surface or subsurface orientation of the target pest. Specific gravity, solubility, hydrolysis, melting point, biodegradation, photodegradation, and reactivity interactions of the coating(s), carrier, and bioactive agent(s) in an admixture formulation were utilized to design specific compositions for aquatic pest-management applications. If the formulation components were properly matched, a Matricap® composition could deliver a bioactive agent to specific surface and/or subsurface areas of an aquatic habitat to target the orientation and/or feeding patterns of vector or pest populations for prolonged periods.

Granular matrix compositions were initially designed for controlled delivery of single-action or joint-action formulations of biolarvicides (e.g., Bacillus thuringiensis var. israelensis and/or Bacillus sphaericus) or biolarvicides and insect growth regulators (e.g., methoprene and pyriproxyfen). Efficacy was demonstrated against vector and nuisance species of mosquitoes. Controlled delivery of bioactive agents used to control mosquito larvae (e.g., Aedes, Anopheles, Culex, and Ochlerotatus spp.) in surface or subsurface areas of a water column was shown to be a function of the type, number, and/or concentration of coating agents incorporated into a granular composition.

Contributed Paper. Thursday, 9:15. (174)

Quantifying the serine protease enzymes of neat gut juice from C. fumiferana (spruce budworm)

Ross Milne, Great Lakes Forestry Centre, Sault Ste. Marie ON

Numerous studies rely on the proteolytic activation and or digestion of both natural and recombinant proteinaceous Bt toxins. More recently reports as to how the larva respond to these toxins show that the proteolytic enzymes change when challenged with the toxins. In the past we and others have purified and variously characterized the proteolytic enzymes in gut juice as to their general activity with artificial substrates. However in order to understand the role of these enzymes in the developing insect gut we need to fully characterize their kinetics. As a first step we have quantified the serine protease activity using burst kinetics. Combined with specific irreversible inhibitors we can both identify the specific serine protease and quantify the catalytic activity in a neat gut juice sample. The application of this technique to other lepidopteran larva will be discussed.

Contributed Paper. Thursday, 9:30. (175)

Bioassay of a highly purified vip 3a toxin against forest pest lepidoptera

Ross Milne, Yeuhong Liu and Kees van Frankenhuyzen, Canadian Forestry Service Sault Ste. Marie

Vip 3a toxin is a soluble insect active toxin that is produced during vegetative growth in some Bt strains including HD-1. The vip 3a toxins are known to influence the overall toxicity for those cultures that produce both the insoluble crystalline delta-endotoxins and the vip toxins. In an effort to understand the contribution of the vip toxins in forest pest lepidoptera we have prepared a highly purified active vip3a protein. Activity was monitored throughout the purification steps using Spodoptera exigua. Bioassay data for Choristoneura fumiferana (spruce budworm) and Lymantria dispar (gypsy moth) was generated for both the vip only, and the delta-endotoxin reconstituted with vip, conditions.

Contributed Paper. Thursday, 9:45. (176)

Authorisation and commercialisation of microbial biopesticides: regulatory innovation and the regulatory state

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Agriculture faces a serious challenge to develop sustainable pest management systems. Microbial biopesticides can make important contributions to IPM, but their commercialisation is affected strongly by the regulatory system that governs their authorisation. This occurs no more so than in the European Union, where arrangements for microbial agents are complex and operate separately at national and EU-wide levels. It is widely accepted that the current system needs to improve. Although there is a strong role for government in helping new industries that bring positive public benefits, the regulatory authority has a difficult job to ensure product quality and public safety while not inhibiting commercialisation. We have been investigating the prospects for regulatory innovation for microbial biopesticides in the UK, although our work is relevant generally. We have used, as a starting point, the debate about Weberian bureaucratic theory and Moran's theory of The Regulatory State combined with empirical research based on interviews with key actors and a comparison of arrangements in the EU and US. We have developed a set of regulatory design principles that we hope can be used to guide regulators and practitioners to achieve more innovation for the commercialisation and authorisation of microbial pest control agents.

CONTRIBUTED PAPERS, Thursday 8:00 - 10:00

BACTERIA 4

Contributed Paper. Thursday, 8:00. (177)

Identification of the receptor-binding motif of the binary toxin from Bacillus sphaericus

Kamonnut Singkhamanan, Institute of Molecular Biology and Genetics, Mahidol University

The mosquito-larvicidal binary toxin produced by Bacillus sphaericus (Bs) is composed of BinB and BinA subunits with molecular masses of 51 and 42 kDa, respectively. Both proteins function together to kill mosquito larvae as binary toxin. BinB is proposed to act as specific receptor binding component, whereas BinA is important for toxicity. To study the function of amino acids in 2 regions of BinB that are absent in BinA, 4 block mutations (111YLD113=>111AAA113, 115NNH117=>115AAA117, 143GEQ145=>143AAA145, and 147FQFY150=> 147AAAA150) were constructed. Mosquito larvicidal activity assays against Culex quinquefasciatus larvae revealed that 111YLD113=>111AAA113, 115NNH117=>115AAA117, and 143GEQ145=>143AAA145 caused slightly reduction in toxicity comparing to that of the wild type, whereas replacement at 147FQFY150=> 147AAAA150 resulted in total loss of toxicity. To identify residues playing critical role in this region, single amino acid substitutions were performed. Mosquito larvicidal activity assays revealed that two mutant toxins (F147A and Q148A) showed less toxicity than the wild type. However, the mutants F149A and Y150A yielded total loss of toxicity. Intrinsic fluorescent spectroscopy analyses suggest that all mutant proteins should have similar structures to that of the wild type. It is possible that F149 and Y150 residues play an important role for receptor binding of BinB. The receptor binding assay of both mutant toxins comparing to the wild type toxin is under investigation.

Contributed Paper. Thursday, 8:15. (178)

Molecular tools for detection and monitoring of an allele conferring Bacillus sphaericus resistance in Culex quinquefasciatus

Chalegre, K.D.M.1, Amorim, L.B.1, Oliveira, C.M.F.1, Romão, T.P.1, de Melo-Neto, O.P.2, Silva-Filha, M.H.N.L.1
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The insecticidal action of the entopathogen Bacillus sphaericus (Bsp) towards Culex quinquefasciatus larvae depends on the binding of the binary toxin to a midgut membrane-bound receptor, the α-glucosidase Cqm1. A resistance mechanism previously described is related to a 19-nucleotide deletion in the cqm1 gene, which prevents the expression of the receptor on epithelium. The goal of this work was to develop tools to identify the alleles cqm1/cqm1-d19 and to detect the Cqm1 expression, using individual 4th instar larvae from a susceptible and a Bsp-resistant colony. PCR reactions with primers flanking the 19-nucleotide deletion in the cqm1 gene were performed, and in gel α-glucosidase assays to detect the receptor expression were done. The size and sequence of the amplified fragments obtained in the assays characterized the susceptible and resistant genotypes. The enzymatic assays showed that resistant larvae did not display the catalytic band corresponding to the α-glucosidase Cqm1, against its presence in samples of susceptible larvae. The genotype and phenotype of larvae samples from susceptible and resistant colonies were established. Those tools have been used for monitoring the frequency of this resistance allele in samples of Bsp-treated and non-treated populations, from urban areas of Recife, Brazil.

Key words: allele frequency; α-glucosidase; vector control; binary toxin; Cqm1; receptor.

Contributed Paper. Thursday, 8:30. (179)

Recombinant bacteria delay the evolution of resistance in mosquito larvae

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To reduce the risk of resistance, two recombinant strains of Bacillus thuringiensis that synthesize different combinations of mosquitocidal proteins from Bacillus sphaericus (Bs), Bacillus thuringiensis subsp. israelensis (Bti) and/or B. thuringiensis subsp. jegathesan (Btj) were genetically engineered for high activity and resistance management against susceptible and resistant larvae of Culex quinquefasciatus and species of the Anopheles gambiae complex. One

strain, Bti/Bs, consisted of Bti IPS-82 engineered to produce eight-fold the amount of Bs Bin from Bs2362, whereas the second strain (Bs11BcytA) produced Bti Cyt1Aa, Btj Cry11Ba, and Bs2362 Bin. To test the capacity of these strains to delay resistance, C. quinquefasciatus colonies were selected with each recombinant strain. After 20 generations, the Bti/Bs-selected colony showed only a 5-fold decrease in susceptibility, whereas the colony selected with Bs11BCytA showed higher resistance, 17–18 fold. Interestingly, susceptibility was more variable in the latter colony, and the higher resistance was not sustained under continuing selection. Given the intense pressure characteristic of laboratory selection, these results indicate that recombinant bacteria that produce various combinations of mosquitocidal proteins, particularly those that contain Cyt1A, can be used to reduce costs and delay the evolution of resistance under field conditions.

Contributed Paper. Thursday, 8:45. (180)

Interactions between ORF157, ORF156 and the iteron in replication of pBtoxis of Bacillus thuringiensis subsp. israelensis

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We identified a mini-replicon of pBtoxis from Bacillus thuringiensis subsp. israelensis that contains an operon encoding two proteins (ORF156 and ORF157), both of which are required for replication. Although these proteins share no homology with known plasmid replication (Rep) proteins, the ORF157 contains a helix-turn-helix motif, common among these, and ORF156 contains the signature motif present in FtsZ/tubulin-like proteins, proteins known to function in cell division. Here we show that the replication origin of pBtoxis is located in a 97-bp fragment, upstream of the pBt157 gene, which contains 48-bp direct-repeat sequence (iterons) and a DnaA box motif. Based on result using recombinant proteins, we demonstrate that rORF157, but not rORF156, binds specifically to the iteron sequence, suggesting that ORF157 functions as a Rep protein. Although rORF156 did not bind to the iteron sequence, it bound to the ORF157-DNA complex in vitro, as determined by electrophoretic mobility shit assays (EMSA). This suggests that direct interaction between ORF156 and ORF157 is required for pBtoxis replication. Moreover, we show that rORF156 has GTPase activity characteristic of the FtsZ/tubulin superfamily of proteins. Overall, our results suggest that ORF157 and ORF156 are involved in initiation of pBtoxis replication, and possibly in the partitioning of this plasmid during sporulation.

Contributed Paper. Thursday, 9:00. (181)

Economical Overproduction of Bioinsecticides of Bacillus Thuringiensis by Random Mutagenesis, Heat and Salt Stress, Control of Oxydative Metabolism and Adequation of Fermentation Technology

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Bioinsecticides based on preparations of spores and insecticidal crystal-proteins (ICPs) produced by the bacterium Bacillus thuringiensis (Bt) proved to be a high tool for fighting some agricultural pests and vectors of diseases. However, the use of Bt preparations as commercial insecticides would be prohibitively expensive because it is not easy to reach cheap overproduction of ICPs during large-scale fermentation. Here, we report possibilities to improve delta-endotoxins production by improvements of Bt strains through random mutagenesis. Interestingly, we obtained mutants, which exhibited high yields of ICPs by sporulating cells in cheap media already developed in the laboratory. Moreover, high improvements of ICPs production were obtained as a consequence of responses of Bt strains to low levels of heat and salt stress. Each stressor results differently in the improvement of delta-endotoxins production, but both were shown to be most efficient at the beginning or the mid-exponential phase of the cultures which become resistant at the stationary or the sporulation steps. Heat stress caused increase of 84% of synthesis yields of the sporulating cells, In contrast, salt caused increase of 25% of spores counts, corresponding to 28% toxins production improvement. Combined effects of both stressors lead to toxins production improvement of 66% , yield improvement of 40%. We focused on the overcome of carbon repression catabolite, closely related to oxidative metabolism, by an adequate control of dissolved oxygen in the cheap media we formulated for Bt insecticides production. We showed that an equilibrium between the high density of vegetative cells and their ability to synthesize toxins during their sporulation was necessary to take into account. 40% increase of ICPs production was reached into 3 l fermenter Combination of mutagenesis, heat and salt stress and oxidative metabolism control allowed more than 100% improvement of delta-endotoxins. These results are of great importance in practical point of view, since high bioinsecticides production was reached in an optimised fermentation technology taking into account all the improvements developed in the study.

Contributed Paper. Thursday, 9:15. (182)

Changes in the transcriptional profile of Helicoverpa armigera after feeding with Cry1Ac or Cry2Ab Bacillus thuringiensis toxins.

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Helicoverpa armigera is one of the most important insect pests in many cotton-producing countries, including Australia, India, and China. In recent years, transgenic cotton expressing simultaneously the Bacillus thuringiensis (Bt) Cry1Ac and Cry2Ab toxins are been extensively used for the efficient control of this pest.

The response of insects to Bt toxin involves a change in the expression of a large number of genes. Understanding the reaction of the insect to pathogen or toxin attack might improve the efficacy of Bt-based products. Additionally, knowing the genes involved in the response can highlight those pathways that may be altered in insects showing resistance.

In our current work, a cDNA-microarray from the midgut of H. armigera has been employed for the analysis of the insect response to sublethal doses of the Cry1Ac as well as to the Cry2Ab toxin. Of the genes analysed, around 3% showed differential gene expression after Cry1Ac or Cry2Ab treatment. The affected genes include a broad range of protein families such as: detoxification and digestive enzymes, cellular signalling, and immune response, among others. Overall, there were similar patterns of gene expression changes for the two toxins, but a few genes showed changes unique to an individual toxin.

Contributed Paper. Thursday, 9:30. (183)

Effects of amino acid substitutions in a highly conserved region of a cytolytic toxin from Bacillus thuringiensis

Boonhiang Promdonkoy1, Jureeporn Cheurdaungphoi1, Patcharee Promdonkoy1, Amporn Rungrod1, Wanwarang Pathaichindachote1, Mongkon Audtho1 and Sakol Panyim2

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Cyt2Aa2 is a cytolytic and mosquito-larvicidal protein produced by Bacillus thuringiensis subsp. darmstadiensis. Multiple sequence alignment revealed that amino acid sequence "TFTNL" in the loop connecting alpha-D and beta-β4 is highly conserved in all Cyt toxins. Alanine substitution was employed to investigate the role of each residue in this region. All mutant proteins were produced as inclusion bodies at high level comparable to that of the wild type. However, inclusion bodies of F143A and L146A mutants were unable to solubilize in carbonate buffer. Result suggested that substitutions at both positions affect protein folding and crystal packing. The mutant N145A was completely lost its activity although it could yield the protease-resistant core after digestion with proteinase K similar to that of the wild type. Substitution at this position should not have any effect on protein folding or crystal packing but may affect conformational changes of the protein during membrane binding or oligomerization. Replacements of Threonine by Alanine (T142A and T144A) did not affect expression, solubilization and protease activation but altered specificity of the toxin. Haemolytic activity against sheep RBC was reduced for both mutants. While the mutant T142A exhibited high toxicity to Culex but not Aedes larvae, T144A showed high toxicity to both larvae comparable to that of the wild type. Our results suggesting that amino acids in a highly conserved region "TFTNL" are involve in protein folding, solubilization and specificity of the Cyt toxins.

Keywords: Bacillus thuringiensis, Cyt toxin, mosquito, mutagenesis, toxicity

Contributed Paper. Thursday, 9:45. (184)

Effects of mutagenic residues at N- and C-termini on structure and function of a cytolytic toxin from Bacillus thuringiensis

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Cyt2Aa2 is a cytolytic and mosquito-larvicidal protein produced from Bacillus thuringiensis subsp. darmstadiensis. To obtain an active toxin, proteolytic processing of both termini is required. Structural analysis suggesting that interaction between both ends could prevent the protoxin to exhibit its activity. To reduce this interaction, H-bond between Asp39 and Asn241 was eliminated by replacing Asp39 with Leu (D39L). In order to completely abolish interactions between N- and C-termini, the truncated toxin lacking 31 amino acids at C-terminal was generated by substitution of Ser229 codon with stop codon (S229stop). The D39L mutant showed similar expression and solubility to the wild type but the S229stop mutant showed lower expression and solubility. The D39L toxin retained biochemical properties and tertiary structure to those of the wild type but the S229stop mutant showed a complete lost of larvicidal activity. Hemolytic activity assay revealed that S229stop protoxin could not be able to lyse erythrocytes, whereas its activated form was hemolytic. Intrinsic fluorescent spectroscopy analysis indicated that tertiary structure of the S229stop toxin is different from the wild type. Results indicated that H-bond between Asp39 and Asn241 does not involve in toxin inactivation and amino acids in C-terminus are required for protein folding, expression and solubilization.

FUNGI DIVISION SYMPOSIUM, Thursday, 14:00 - 16:00

Fungal Secondary Metabolites: Knowns and Unknowns

Symposium. Thursday, 14:00. (185)

Terrequinone A biosynthesis – implications beyond Aspergilli

Dirk Hoffmeister

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LaeA, a global transcription regulator for secondary metabolism in Aspergilli, was employed for microarray-based screening of the model fungus Aspergillus nidulans genome for actively transcribed natural product genes (Bok et al., 2006, Bouhired et al., 2007). Among those, the tdiA-E biosynthetic gene cluster for terrequinone A was identified. After heterologous expression and biochemical characterization of the enzymes catalyzing backbone assembly - the transaminase TdiD and quinone synthetase TdiA – the terrequinone core structure was synthesized in vitro.

Homologs of tdiA (in most cases coupled to tdiD homologs) were found in the genomes of numerous microbes, ranging from plant pathogenic bacteria to saprobic or symbiotic filamentous fungi. Therefore, TdiA and TdiD, and their corresponding genes, serve as a blueprint for natural product enzymes/genes in a variety of microorgamisms and may help identify more unknown biosynthetic abilities. Implications for chemical ecology, as well as drug discovery, will be presented.

Symposium. Thursday, 14:20. (186)

Integration of polyketides into the life cycle of Fusarium graminearum.

Frances Trail, Department of Plant Biology, Michigan State University, East Lansing, MI 48824.

Type I Polyketide Synthases (PKSs) are multidomain enzymes responsible for synthesizing a gamut of compounds with varied functions. From the genomic sequence of the filamentous fungus Fusarium graminearum, we identified fifteen putative polyketide synthase genes. We have disrupted each of these genes and individual disruption mutants have been analyzed for traits such as vegetative growth, mycotoxin and pigment production, perithecium production, ascospore discharge and pathogenicity. From these analyses, we have identified PKS genes responsible for biosynthesis of several mycotoxins and 2 pigments involved in the life cycle. Expression analysis of these genes under varied culture conditions revealed that they are differentially expressed. Our work is now focused on assigning functions to the remaining PKS genes and elucidate their role in the life cycle of this important wheat pathogen.

Symposium. Thursday, 14:40. (187)

Using molecular genetics to reveal metabolic pathways of Metarhizium anisopliae

Churchill, A.C.L.

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Entomopathogenic fungi are rich producers of secondary metabolites, many of which have been proposed to play roles as insect toxins. One prominent class of secondary metabolites is the nonribosomal peptides, of which destruxin from Metarhizium anisopliae is well described. Nonribosomal peptides are synthesized by large, multifunctional enzymes called nonribosomal peptide synthetases (NPS). An NPS from M. anisopliae, MaNPS1, was disrupted by Agrobacterium tumefaciens-mediated transformation. MaNPS1 gene knockout (KO) transformants exhibited in vitro development, responses to external stresses, virulence against insect hosts, and levels of destruxin production comparable to an ectopic transformant and the wild type strain. Chemical analyses revealed the presence of a novel peptide in extracts of control strains of the fungus, whereas

the compound was undetectable in MaNPS1 KO strains treated identically. This is the first report of targeted disruption of a secondary metabolite gene in M. anisopliae, which revealed the presence of a compound not reported previously from fungi. Expression of additional genes predicted to be involved in secondary metabolite synthesis or efflux has been detected in infected insects. These studies suggest additional targets for functional analyses examining the biological roles of secondary metabolites in insect pathogenesis.

Symposium. Thursday, 15:00. (188)

New secondary metabolites from Metarhizium anisopliae

Stuart B. Krasnoff

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A knockout study of a nonribosomal peptide synthetase (NPS) gene from Metarhizium anisopliae revealed natural products that are either entirely novel or new for the species. One of the NPS knockout mutants generated in the study overproduces NG-391 and NG-393, desmethyl analogs of fusarin C and 8-Z-fusarin C, which are well-known mutagenic mycotoxins from Fusarium spp. affecting corn. Like the fusarins, the NG-39x compounds show potent S-9 mutagenic activity in the Salmonella mutagenicity test. A survey of M. anisopliae strains derived from commercial biocontrol products showed significant variation in the amounts of these compounds produced in vitro. NPS knockouts from the same study failed to produce a family of novel cyclic peptides that were isolated from the wild type. We also report the production by M. anisopliae of yet another peptide that has been previously identified as an NPS product in several fungal species. The discovery of these natural products, and remaining questions about how they serve the producing organism, have implications for risk assessment of biocontrol microbes and illustrate the importance of finding new ways to search for fungal secondary metabolites.

Symposium. Thursday, 15:20. (189)

Risk assessment of metabolites produced by entomopathogenic fungi – a REBECA statement

Hermann Strasser1 & Ralf-U. Ehlers2

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In April 2007 a proposal on "the Risk Assessment of Metabolites produced by Micro-organisms in Plant Protection Products" was presented to the European Commission, EU member states, national and international organisations (i.e. BPSG, EFSA) in order to facilitate the registration procedure for plant protection products containing microbials as the active ingredient. This work was initiated by a working group in the REBECA Policy Support Action (SSPE-CT-2005-022709). It aims at a facilitation of the procedure for Annex I inclusion and at a facilitation of national registrations. Micro-organisms used as active substances in plant protection products in the EU are regulated according to the EU Council Directive 91/414/EEC. Data requirements for the registration of micro-organisms as active substances and of products based on micro-organisms are laid down in the Council Directive 91/414/EEC, amended by the Commission Directive 2001/36/EC. The Uniform Principles for evaluation and authorisation of plant protection products containing micro-organisms are laid down in the Council Directive 2005/25/EC.

New insights into risk assessment of metabolites produced by entomopathogenic fungi in Europe are given and will be discussed in the symposium "on fungal secondary metabolites".

CONTRIBUTED PAPERS, Thursday 14:00 - 16:00

MICROBIAL CONTROL 4

Contributed Paper. Thursday, 14:00. (190)

The susceptibility of Anopheles mosquitoes to Bacillus thuringiensis subsp. israelensis

Irene Ketseoglou1, Maureen Coetzee2, and Gustav Bouwer1

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Two of the species (A. gambiae sensu stricto and Anopheles arabiensis) in the Anopheles gambiae sensu lato species complex are widely distributed and important vectors of human malaria in sub-Saharan Africa. In contrast, Anopheles melas and Anopheles merus have only regional importance as malaria vectors. Anopheles funestus, which is not a member of the A. gambiae complex, is a widely distributed and important malaria vector. Some species in the A. gambiae complex, such as Anopheles quadriannulatus, are not malaria vectors. Several populations of A. gambiae, A. arabiensis, and A. funestus are showing resistance to one or more of the insecticide classes used in vector control programmes. As part of a biological control programme, the susceptibility of A. funestus and members of the A. gambiae complex to Bacillus thuringiensis subsp. israelensis (Bti) were evaluated. On the basis of concentration-response bioassays using Bti spore-crystal suspensions, A. funestus larvae were the least susceptible and A. quadriannulatus larvae the most susceptible to Bti. When compared to A. quadriannulatus (species A), the resistance ratios for A. merus, A. arabiensis, and A. funestus were 1.2, 1.2, and 1.5 respectively. The results confirm the potential of Bti as a control agent of Anopheles malaria vectors.

Contributed Paper. Thursday, 14:15. (191)

Species identification and host range testing of a new entomopathogenic member of the Enterobacteriaceae

Mark RH. Hurst1, Sandra Young1, Tracey Nelson1, Trevor A. Jackson1, Anette Becker2 & Travis R. Glare1

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During routine collection and processing of the larvae of the New Zealand grass grub, Costelytra zealandica (Coleoptera: Scarabaeidae) for use in bioassays, many larvae succumbed to an apparent disease. Screening of the dead larvae for the presence of pathogens revealed a bacterium present in all exhibiting symptoms of shiny brown cadavers. Healthy larvae were refed with the isolated bacteria and the symptoms were repeated. Some fed larvae succumbed to a rapid disease onset, turning an amber colour within 4 -16 hours post ingestion, followed by a progressive browning leading to a blackened deliquescent state and mortality within 24-72 hours post infection. Subsequent studies demonstrated that the bacterium has a broad spectrum activity affecting Coleoptera, Lepidoptera and members of other insect orders. Phylogenetic analysis in conjunction with DNA-DNA hybridisation studies demonstrate that this is a new species of bacteria falling within the family Enterobacteriaceae and the Genus Yersinia. The paper concerns the isolation, pathology and host range testing of the unique species of Yersinia

Contributed Paper. Thursday, 14:30. (192)

A novel Bacillus thuringiensis isolate that produces cuboidal crystals and is highly toxic to larvae of Trichoplusia ni

Izabela Swiecicka,1 Dennis K. Bideshi,2 and Brian A. Federici2

Department of Microbiology, Institute of Biology, University of Bialystok, Bialystok, Poland1 and Department of Entomology, Uni-

versity of California, Riverside, Riverside, California 92521, USA2
A new isolate of Bacillus thuringiensis, IS5056, was obtained from soil collected in northeast Poland. This isolate synthesized large cuboidal crystals during sporulation, and was highly toxic to larvae of Trichoplusia ni, with LC50s of 16.9 and 29.7 μg/ml of diet to, respectively, second and fourth instars. SDS-PAGE analysis of purified crystals showed that this isolate produced a crystal protein of approximately 130 kDa. MALDI-TOF sequence analysis of this protein revealed that it belonged to the Cry1 type. Using primers based on cry1A, a 3.9 kb fragment was amplified by PCR. Nucleotide sequence analysis showed that the IS5056 δ-endotoxin gene was most closely related to cry1Ac. Southern blot analysis indicated that this gene was located on a large plasmid in IS5056. Expression of this gene in B. thuringiensis 4Q7 using the E. coli–B. thuringiensis shuttle vector pHT3101 yielded a crystal protein identical in mass to that produced by IS5056. In addition, comparative analysis using gyrB and 16s rRNA sequences suggested that IS5056 is a new entomopathogenic serotype of B. thuringiensis.

Contributed Paper. Thursday, 14:45. (193)

Quasi-innate-immunity: hemolymph peptide induction with Bacillus thuringiensis (Bt) exposure and bacterial challenge in Bt-resistant and susceptible cabbage loopers, Trichoplusia ni (Hubner).

Ericsson, J.D1. Janmaat, A2. Myers, J.H3. Lowenberger, C1.
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Greenhouse populations of cabbage looper have developed resistance to Bacillus thuringiensis kurstaki. This resistance has been shown to be due in part to a physiological incompatibility between Bt toxins and the midgut brush border membranes they bind. In the absence of Bt, selection against resistant genotypes causes the refractory mutation to decline in frequency with subsequent generations. Other factors, including components of the innate immune response may also reduce the toxicity of Bt. We have initiated studies into the expression profiles of hemolymph and midgut proteins in Bt-resistant (Bt-R), and Bt-susceptible (Bt-S) loopers, exposed to Bt to determine if innate immune responses contribute to the refractory trait of Bt-R. In addition we have evaluated the profile of innate immune molecule expression in Bt-R and Bt-S insects after challenge with an injection of Gram negative bacteria into the hemocoel to simulate the secondary sepsis known to occur with midgut degradation. Differentially expressed peptides are being characterized in terms of antimicrobial activity against bacteria and fungi.

Contributed Paper. Thursday, 15:00. (193,1)

cDNA microarray analysis of genes involved in parasitization of the silkworm Bombyx mori by tachinid parasitoids

Andrew Kalyebi1, Y. Nakamura1, K. Mita1, H. Noda1, R. Ichiki2, S. Nakamura2 and K. Kadono-Okuda1
1National Institute of Agrobiological Sciences, Tsukuba, Japan.
2Japan International Research Center for Agricultural Sciences, Tsukuba, Japan.

Insect hosts are continuously exposed to potentially pathogenic microorganisms and eukaryotic parasites. In the silkworm Bombyx mori, immune responses such as encapsulation, phagocytosis and antimicrobial proteins have been well studied. However, very little is known about the parasitic effects or host-parasite relationships resulting from parasitization by tachinid flies.

With this objective, we used B. mori parasitized by three tachinid species, Exorista japonica, Drino inconspicuoides and Pales pavida, which exhibit different oviposition strategies. Hemocytes, fat body and silk gland were collected from B. mori and their RNAs were used to analyze gene expression by cDNA microarray. Analysis of data is being undertaken to identify the genes. Preliminary results in two species (Exorista japonica and Drino inconspicuoides) indicate a completely different set of genes being up-regulated or down-regulated. We will compare gene expression profiles of parasitized and non-parasitised B. mori to identify genes whose expression levels change following parasitization. These studies will elucidate the pathological and defensive mechanism of the host at the molecular level and will highlight the common and specific aspects of gene expression cascades between health and diseased states.

Contributed Paper. Thursday, 15:15. (195)

Seasonal migration, local movement and the patterns of Bt resistance in cabbage looper populations in British Columbia

Michelle Franklin1, Alida Janmaat2 and Judith Myers1
1Dept. Zoology, University of British Columbia, Vancouver, Canada
2Biology Department, University College of the Fraser Valley, Abbotsford, Canada

Most of the interest in the movement of moths exposed to the microbial insecticide, Bacillus thuringiensis has been in relation to the use of refuges for resistance management in transgenic crops that express Bt toxins. In British Columbia elevated levels of Bt resistance occur in cabbage loopers in vegetable greenhouses but surprisingly, resistance apparently spreads from looper populations selected through high use of Bt sprays, to greenhouses where Bt has not been sprayed. Field populations of cabbage loopers in California and Oregon that are potential sources of seasonal migrants to British Columbia, are highly susceptible to Bt as are field populations in British Columbia. There is no evidence however that these susceptible field populations serve to dilute the levels of resistance of greenhouse populations through immigration during the summer. The persistence and spread of Bt resistance among greenhouse populations of cabbage loopers will be discussed in light of the cost of resistance and the incomplete dominance of resistance expression in cabbage loopers feeding on cucumbers.

Contributed Paper. Thursday, 15:30. (196)

Response of Heliothis virescens to different diets containing the same amounts of Bacillus thuringiensis Cry1Ac

Carlos A. Blanco1 and Fred Gould2
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Abstract: Heliothis virescens (tobacco budworm, Lepidoptera: Noctuidae) has been the target of genetically-modified cotton (Bt cotton) that produces B. thuringiensis toxins. The adoption of this agricultural biotechnology could be jeopardized by the development of B. thuringiensis-resistance in targeted pests. Therefore, several tactics have been implemented in order to impede that pests become resistant to Bt cotton. One of them consists on screening field-collected pests for their response to B. thuringiensis as an early indicator of potential problems in areas where Bt cotton adoption is high. Several laboratories might perform the screening of the same pest, and because methods are expected to vary among laboratories, it is important to understand the role of insect artificial diet in the potential different response of H. virescens to Cry1Ac-incorporated diet. We tested the response of four different tobacco budworm colonies with four different diets containing the same amount of Cry1Ac finding significant variation among diets. Specific results will be discussed in the presentation.

Contributed Paper. Thursday, 15:45. (197)

Verticillium lecanii (Lecanicillium spp.) as plant bodyguards

Masanori Koike and Daigo Aiuchi*
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It is well known that entomopathogenicVerticillium lecanii (Lecanicillium spp.) can control not only insect pests (aphids, whitefly, thurips), but also plant diseases (rust and powdery mildew). We bred good V. lecanii strains as plant bodyguards using protoplast fusion (Aiuchi et al. 2004; Aiuchi et al. 2007) . Firstly, we selected three epiphytic strains from Japanese isolates of V. lecanii for dual (insect and disease) biological control agents (BCAs). These strains were highly epiphyte on cucumber and tomato plants, and good BCAs. Two strains (A-2, B-2) could control aphids, whitefly, powdery mildew and fungal wilt disease (Fusarium oxysporum and Verticillium dahliae). Secondly, one (B-2) of three strain fused with Mycotal® and Vertalec®. by PEG method. Finally, we selected several good strains as BCAs for insect pests (aphids and whitefly), powdery mildew and soybean cyst nematode. We will report on the result that these good strains could play an important role as plant bodyguards.

CONTRIBUTED PAPERS, Thursday 14:00 - 16:00

VIRUSES 5, Insect Virus Diversity and Evolution

Contributed Paper. Thursday, 14:00. (198)

The genes driving baculovirus genome evolution

Elisabeth A. Herniou1, Hilary A. M. Lauzon2, Alejandra Garcia-Maruniak3, Basil M. Arif2, James E. Maruniak3 and Paolo M. A. Zanotto4
1 Division of Biology, Imperial College London, Silwood Park, Ascot, UK; 2 Canadian Forest Service, Great Lakes Forestry Centre, Sault Ste. Marie, Canada; 3 Entomology and Nematology Department, University of Florida, Gainesville, USA ; 4 LEMB – Instituto de Ciências Biomédicas - São Paulo, Brazil

Coevolution plays an important role in the adaptability of baculoviruses to their hosts. Fine-tuning of these interactions is greatly enhanced by the fluidity of their genomes, with the capture of specific genes enhancing their fitness. However, if some genes provide clearly linked functional benefits, the majority of genes contained within viral genomes do not. It ensues that we have little knowledge of which genes or groups of genes have been instrumental in the diversification of the family Baculoviridae. Here we test new phylogenetic methods based on complete genome analyses. The resulting trees are then compared to the now traditional method of using a concatemer of the 29 genes common to all baculoviruses. Laslty, we perform a detailed analysis of the evolution of gene content. Altogether this will give us new insights on the genes driving the evolution of baculoviruses.

Contributed Paper. Thursday, 14:15. (199)

Trichoplusia ni and Chrysodeixis chalcites single nucleopolyhedroviruses: Genomic and biological comparison.

Martin A. Erlandson1, Monique M. van Oers2, David A. Theilmann3, Just M. Vlak2
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Trichoplusia ni single nucleopolyhedrovirus (TnSNPV) was originally isolated from T. ni larvae feeding on cole crops. TnSNPV appears to have a narrow host range and is currently under investigation as a potential viral pesticide for T. ni control in vegetable greenhouses in Canada. Chrysodeixis chalcites nucleopolyhedrovirus (ChchNPV) was recently isolated from larvae of C. chalcites, a major pest in Dutch greenhouses. The complete genome sequences of each virus were recently published and indicate they are closely-related group II NPVs with virtually collinear arrangements of ORFs. The two virus genomes also are unique among NPVs in that they each have homologues of class II cyclobutane pyrimidine photolyases involved in light activated DNA repair, and no typical baculovirus hr sequences were detected in either virus. The ChchNPV genome is 149,622 bp containing 151 predicted ORFs while TnSNPV is 134,394 bp containing 144 predicted ORFs and there are a number of ORFs unique to each virus. Both viruses are infectious for T. ni but in dose response assays in 4th instar T. ni, TnSNPV had an LD50 value of 8.2 (4.5-13.6) PIB/larva while ChchNPV was significantly less infectious, LD50 of 36.6 (18.7-67.1) PIB/larva. When infected with biologically equivalent LD90 doses, TnSNPV had an ST50 value of 143.6 hours pi where as ChchNPV took significantly longer, ST50 value of 232.8 hours pi, to kill 4th instar T. ni. The gene content comparisons of the two viruses are addressed in the context of potential virulence and host specificity differences.

Contributed Paper. Thursday, 14:30. (200)

Towards the complete genome sequence of the baculovirus-related nonoccluded Oryctes rhinoceros nudivirus of beetles

Yongjie Wang 1, Regina G. Kleespies2, Monique van Oers3, Barry Ale4, Moslim B. Ramle5,
Trevor Jackson6, Just Vlak3, Johannes Jehle1
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2 Federal Biological Research Center of Agriculture and Forestry, Institute for Biological Control, Darmstadt, Germany
3 Laboratory of Virology, Wageningen University, Wageningen, the Netherlands
4 Crops Division, Nuu, Ministry of Agriculture and Fisheries, Samoa
5 Malaysian Palm Oil Board, Kuala Lumpur, Malaysia
6 AgResearch, Lincoln, New Zealand

The Oryctes rhinoceros nudivirus (OrNV) was discovered in the 1960s in Malaysia and has been effectively used to control the rhinoceros beetle, Oryctes rhinoceros (Order Coleoptera), in coconut and oil palm in Southeast Asia and the Pacific. The virus contains enveloped, rod-shaped and dsDNA virions, and replicates in the nuclei of infected midgut and fat body cells. Recently, PstI C and D fragments covering about 30% of OrNV genome have been cloned and sequenced. Comparative genomic analysis indicated that OrNV is genetically most closely related to Gryllus bimaculatus nudivirus (GbNV) infecting the fat body cells of the cricket G. bimaculatus (Order Orthoptera), and to Heliothis zea nudivirus 1 (HzNV-1), which causes a persistent viral infection in the IMC-Hz-1 cell line isolated from the adult ovarian tissues of the corn earworm H. zea (Order Lepidoptera). The limited availability of OrNV prompted us to devise an alternative strategy for the whole genome sequencing of this virus. Starting from a few infected rhinoceros beetles, the genome of OrNV was comprehensively amplified using 'multiple displacement amplification'. The sequencing of the entire 128 kbp genome of OrNV is being completed. Genomic sequence, gene content, gene order, and phylogenetic analyses of the first coleopteran nudivirus OrNV will be presented.

Contributed Paper. Thursday, 14:45. (201)

Origin of Ichnoviruses : is there consistent molecular support to the Brian Federici's endosymbiogenic theory?

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The origin of some cellular and microbial mechanisms used by parasitoid wasps to circumvent the defence systems of their insect host and to allow the development of their endoparasitoid larva, is a research field that had retained the interest from the entomologist community since one century. Although the parasitoid wasps belong to a very diversified lineage in terms of species number and biological mechanisms to set parasitism, the physiological functioning of mechanisms involving viruses or virus-like particles (VLP) have been elucidated in few lineages, like that of the Ichneumonidae. Briefly, three main situations were found. First, there are Ichneumonidae species for which no viruses or VLP were found in the fluid injected with the eggs into the parasitized host by the wasp female. The second situation is to date exemplified mainly in works done with the wasp Diadromus pulchellus. This species used a true virus, the ascovirus DpAV4, which once injected in the parasitized host, circumvents the defence sytems and arrests the development of the host. The third situation was the most studied and concerns related Ichneumonidae species belonging to genus such as Campoletis, Glypanteles, Hyposoter and Tranosema. In those species, wasp female injects into their parasitized host, their eggs coated with VLP called ichnoviruses, to ensure functions that are similar to those allocated to DpAV4.

Albeit several Ichnovirus genomes are sequenced and that their vertical mode of chromosomal transmission in wasp was elucidated, the origin of Ichnovirus remains very enigmatic. As early as 1991, Brian Federici was the first to correlate the fact that ichnovirus and ascovirus virions were very strikingly similar in shape. In consequence, Brian Federici proposed that ichnoviruses might endosymbiogenic viruses having evolved in the common ancestor of the Ichneumonidae wasps, in which the genome of a domesticated ascoviruses was integrated. Unfortunately, the absence of conserved genes between ichnoviruses and the genes characterized in the virus genomes sequenced to date, including the three ascoviruses HvAV3e, TnAV2c and SfAV1a, did not bring any support to this theory. One possibility explaining this inability to create links between the world of the true viruses and the ichnoviruses is that there was to date no ascovirus with biological traits similar to those of the ichnoviruses and a genome potentially closely related to that of the ichnovirus ancestor which was sequenced. The perspective to sequence the DpAV4 genome was therefore from this point of view an exiting challenge. Here, we present the results of the DpAV4 sequencing genome project. Pertinent information recovered about ichnovirus and ascovirus evolution will be highlighted, and the consequence of their discovery will be discussed in term of coherence for the virus taxonomy.

Contributed Paper. Thursday, 15:00. (202)

Genome analysis of salivary gland hypertrophy virus (SGHV) reveals a novel large double-stranded circular DNA virus from Glossina pallidipes

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Max Bergoinb
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The salivary gland hypertrophy virus of Glossina pallidipes (GpSGHV) infects several species of tsetse flies. Not only it causes salivary gland hypertrophy symptom but also reduces significantly the fertility of the infected flies. We report here the first complete sequence of the GpSGHV genome. This genome is a double-stranded circular DNA of 189, 751 bp encoding 160 putative proteins. It has an A+T content of 72% and a gene density of one gene per 1.7 kb. Although sharing the same morphological features (enveloped rod-shaped nucleocapsid) as Baculoviruses, Nudiviruses, Ascoviruses and Whispoviruses the GpSGHV differs significantly from these groups of large circular dsDNA viruses at the level of its proteins. In fact, some GpSGHV genes are more homologous to eukaryotic genes than to viral genes. Extensive sequence analysis indicates that the GpSGHV genome displays only weak homologies compared to genes of other viruses, i.e. baculovirus, entomopoxvirus, poxvirus, whispovirus, ascovirus and invertebrate iridescent virus. In addition, most of the GpSGHV putative proteins bear no homology to any known proteins suggesting that SGHV represents a novel group of insect viruses. Determination of the genome sequence of SGHV will facilitate a better understanding of the molecular mechanisms underlying the pathogenesis of this virus.

Contributed Paper. Thursday, 15:15. (203)

Characterization of the Musca domestica salivary gland hyperplasia virus (MdSGHV)

A. Garcia-Maruniak1, J. E. Maruniak1, C. Geden2, and D. G. Boucias1
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The MdSGHV was described initially as a non-occluded, enveloped, rod-shaped, double-stranded DNA virus. Feeding bioassays demonstrated that the virus could be transmitted per os to healthy adult house flies and that the infection was responsible for the salivary gland hyperplasia (SGH) symptoms. Female house flies with symptoms of SGH showed no sign of ovarian development. A similar virus causing symptoms of SGH has been reported in the narcissus bulb fly, Merodon equestris and various species of tsetse flies, Glossina spp. Utilizing Nycodenz® gradients, enveloped virus was purified from hyperplasic glands of infected house flies. Negative staining revealed virions measuring 65 by 575 nm containing a unique spiral grooved surface topology. Analysis of genomic DNA using a combination of end-labeling and restriction fragment analysis revealed a ~117 kbp circular genome. Data from bidirectional sequencing on a shotgun EcoRI library in combination with '454' picosequencing has been used to provide an initial overview of the MdSGHV genome. Sequence analysis of selected open reading frames showed homology to tsetse fly SGHV genes.

Contributed Paper. Thursday, 15:30. (204)

A caspase-like gene from Heliothis virescens ascovirus (HvAV-3e) is not involved in apoptosis but is essential for virus replication

Sassan Asgari
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Ascoviruses (AVs) are double-stranded DNA viruses causing a fatal disease in lepidopteran host larvae. A unique feature of AV infection is cleavage of host cells into membrane bound vesicles containing the virions. A recent study showed that a caspase from Spodoptera frugiperda AV (SfAV) is directly involved in initiation of apoptosis and eventually cell cleavage. Results shown here indicate that Heliothis virescens AV does not induce apoptosis in host cells. HvAV codes for a caspase-like protein but no apoptosis was observed when the gene was expressed in vitro. RNAi studies indicated that the gene is essential for virus replication.

Contributed Paper. Thursday, 15:45. (205)

Two Microplitis demolitor Bracovirus virulence factors, PTP-H2 and Glc1.8, induce apoptosis in insect hemocytes

Suderman, R. J., Pruijssers, A. J., and Strand, M. R.
Department of Entomology, University of Georgia, Athens, Georgia 30602

When the parasitic wasp Microplitis demolitor parasitizes Pseudoplusia includens, it injects polydnavirus, causing granular cells, but not plasmatocytes, to apoptose. Because the polydnavirus does not replicate in the wasp's host, its proliferation depends on the survival of the wasp, in which its genome is integrated. Apparently, granulocyte apoptosis allows the parasitoid egg to evade encapsulation, as granulocytes bind foreign targets and recruit plasmatocytes for capsule formation. Two polydnaviral genes, glc1.8 and ptp-H2, caused massive apoptosis when expressed in hemocyte-like SF-21 cells, resulting in only 8 and 12% viable cells remaining after 20 h, respectively. Glc1.8 is a membrane-bound mucin-like protein, and PTP-H2 is a protein tyrosine phosphatase. Cells expressing Glc1.8 and PTP-H2 displayed classical signs of apoptosis including increased effector caspase activity, decreased mitochondrial membrane potential measured by JC-1 staining, and plasma membrane blebbing forming apoptotic bodies. The apoptotic effect of Glc 1.8 and PTP-H2 was nearly completely abrogated in the presence of the pan caspase inhibitor Z-VAD-FMK. A secreted mutant of Glc1.8 and an inactive PTP-H2 mutant showed no apoptotic effect. Cells expressing Glc1.8 and PTP-H2 in the presence of caspase inhibitor exhibited severe cell cycle arrest compared to controls, suggesting a possible cause of apoptosis.

Virus Division Satellite WORKSHOP. Thursday, 16:15 - 19:30

The biology of polydnaviruses: some unresolved issues

Organizers: Michel Cusson, Bruce Webb, Don Stoltz, Peter Krell

Workshop. Thursday 16:15 (205,1)

The biology of polydnaviruses: some unresolved issues

Don Stoltz
Department of Microbiology and Immunology, Dalhousie University, Halifax, NS, Canada,

This presentation will touch upon a number of unresolved issues of interest to the polydnavirus research community, including (very briefly) the question of where these viruses came from. That said, the talk will consist primarily of a series of polydnavirus vignettes that are of particular interest to the presenter himself, and/or which have frustrated his efforts for a significant period of time (e.g., ichnovirus structure).

Workshop. Thursday 16:30 (205,2)

Challenges in defining the functional roles of related virulence gene variants in polydnaviruses

M. R. Strand
Department of Entomology, University of Georgia, Athens GA 30602, USA

Insects rely upon a well-coordinated innate immune system for protection against invading pathogens and parasites. Larger, multicellular parasites are usually killed by encapsulation which involves attachment of multiple layers of hemocytes to the foreign target. Smaller pathogens, in contrast, are killed by a combination of hemocyte-mediated phagocytosis and humoral defenses. Despite the fundamental importance of these responses, our understanding of their regulation in relation to the counter strategies pathogens use to evade host defense responses is limited. Viruses in the family Polydnaviridae are symbiotically associated with parasitoid wasps and are among the most virulent immunosuppressive pathogens of insects. One characteristic of polydnavirus genomes is the presence of multimember gene families. A key challenge is developing approaches and insights into the functions of different gene family members. Here I describe progress toward this goal in our studies of Microplitis demolitor bracovirus (MdBV). By using gain and loss of function screening strategies combined with targeted bioassays, we have developed insights into the functional roles of several gene family members in disruption of the insect immune response. Overall, these results provide important insight on the evolution of polydnaviruses and also identify key virulence determinants underlying immunosuppression.

Workshop. Thursday 16:45 (205,3)

The biology of polydnaviruses: some unresolved issues

Don Stoltz
Department of Microbiology and Immunology, Dalhousie University, Halifax, NS, Canada

This presentation will touch upon a number of unresolved issues of interest to the polydnavirus research community, including (very briefly) the question of where these viruses came from. That said, the talk will consist primarily of a series of polydnavirus vignettes that are of particular interest to the presenter himself, and/or which have frustrated his efforts for a significant period of time (e.g., ichnovirus structure).

Workshop. Thursday 17:00 (205,4)

The sequencing of the integrated form of CcBV: one locus or several loci?

Annie Bézier1, George Periquet1, Jérôme Lesobre1, Gabor Gyapay2, Sylvie Bernard-Samain2, Catherine Dupuy1, Jean-Michel Drezen1
1 Institut de Recherche sur la Biologie de l'Insecte, UMR CNRS 6035, University of Tours, France ; 2 National Sequencing Center, Génoscope, Evry, France

Recently, several polydnavirus (PDV) genomes have been completely sequenced. The dsDNA circles enclosed in virus particles and injected by wasps into caterpillars appear to mainly encode virulence factors potentially involved in altering host immunity and/or development, thereby allowing the survival of the parasitoid larvae within the host tissues. By contrast they do not appear to contain a viral machinery involved in the production of particles in the wasp ovaries. To determine whether virus genes could be identified in the chromosomal form of the PDVs we have isolated the proviral form of CcBV from a BAC library of wasp larvae. The sequences corresponding to most of the 30 dsDNA circles included in virus particles were already obtained. As suggested by former results most of the chromosomal forms of the circles were found to belong to clusters, only one (C26) being flanked on both sides by wasp DNA. However the viral sequences do not appear to form a single tandem array but are organised in 8 different clusters. Previous in situ hybridization studies suggest that three clusters are located on the same chromosomal region thus we are testing new probes to determine whether all viral sequences form a single locus that would comprise several megabases. This study provides important new data on the proviral form of CcBV but still do not allow the identification of the genes involved in virus particle's production.

Workshop. Thursday 17:15 (205,5)

Genomic organisation of the ichnovirus HdIV

Provost B, Samain S, Gyapay G., Drezen JM and Volkoff AN.
Biologie Intégrative et Virologie des Insectes (BIVI), UMR1231 INRA - Université Montpellier II, Montpellier, France

Polydnavirus genomes rely on two distinct forms to ensure their transmission. Indeed, they are maintained in the associated wasp as a provirus from which are generated the multiple circular DNA molecules contained within the virus particles. The genomic organisation of the integrated form, as well as the mechanisms by which the segmented genome is produced, are still poorly known, in particular for the polydnaviruses associated with ichneumonid wasps (Ichnoviruses). We therefore have recently partially sequenced the integrated form of

the ichnovirus HdIV associated with the wasp Hyposoter didymator. Our first results, that will be presented here, suggest that ichnoviruses differ in the genomic organisation of their integrated form from what is described in bracoviruses.

Workshop. Thursday 17:30 (205,6)

Polydnaviruses and virus-like particles as wasp extended genotypes and phenotypes

Sassan Asgari, School of Integrative Biology, University of Queensland, St Lucia QLD 4072, Australia

Polydnaviruses and virus-like particles are evolutionary adaptations of parasitoid wasps to ensure successful development of their progeny inside their hosts. Proteins that constitute virus-like particles devoid of nucleic acids are mainly eukaryotic in origin and not related to viruses; perhaps involved in disrupting cell signalling pathways. The Venturia canescens/Ephestia kuehniella parasitoid-host system is unique among other systems in that the cellular defence capacity of the host remains virtually intact after parasitization. VcVLP particle proteins will be discussed with an insight into the evolution and origin of PDVs and VLPs in the context of extended genotype and phenotypes of the parasitoid wasps.

IFENSB Session IV, Thursday 16:15 - 19:30

ECOLOGY

Symposium. Thursday, 16:15. (206)

Entomopathogenic nematodes in heterogeneous soils: foraging behavior and infection decisions.

GN Stevens, EE Lewis

Department of Nematology, University of California, Davis

Detailed understanding of entomopathogenic nematode (EPN) behaviors may contribute to their effectiveness as biological control agents. This understanding may also prove to be an important component of research on the role of EPNs in soil food webs and model systems. Thousands of IJs emerge from a single infected host and these IJs are confronted with a heterogeneous soil environment. While the distribution of insect hosts in the soil is known to be patchy in general, perception of host cues may be influenced by other cues in the soil, such as those produced by respiring roots, damaged roots, or soil organic matter. There is limited information regarding how these biotic and abiotic cues interact to drive nematode dispersal and foraging behaviors. This talk will focus on recent research that examines IJ foraging and infection decisions in response to a range of biotic and abiotic cues. Our results point to interesting behavioral patterns that may reinforce soil heterogeneity.

Symposium. Thursday, 16:35. (207)

Host behavioral response

Albrecht M. ¬Koppenhöfer

Dept. Entomology, Rutgers University, Blake Hall, 93 Lipman Dr., New Brunswick, NJ 08901

In order to successfully infect a host, entomopathogenic nematode (EPN) infective juveniles (IJs) the IJs have to locate a potential host, attach to its cuticle, penetrate, and establish in the host's body cavity. Soil-dwelling insect and EPN have coevolved with the insect developing barriers to EPN infection and the EPN finding ways to overcome these barriers. These interactions have been particularly well studied in white grubs, the root-feeding larvae of scarab beetles. The first step in the infection process, detection of a potential host, may be made more difficult through the white grubs' tendency to release CO2 in bursts rather than continuously. CO2 is an important volatile host cue for entomopathogenic nematodes. Nematodes that have located a white grub and attached to its cuticle may be eliminated by the grub's aggressive grooming behaviors. These behaviors include rubbing with an abrasive raster situated on the ventral end of the abdomen, brushing with legs or mouthparts, and scraping and chewing motions with their mandibles. In addition, white grubs evade nematode attack by moving away from the nematodes. I will discuss these behaviors and how they vary with white grub species and attacking EPN species.

Symposium. Thursday, 17:55. (208)

Soil food webs, entomopathogenic nematodes, and biological control: shedding some light on an old black box

Robin J. Stuart and Larry W. Duncan

University of Florida, IFAS, Citrus Research and Education Center, Lake Alfred, FL, 33850 USA

Augmentative strategies for the use of entomopathogenic nematodes (epns) in biological control are often developed and implemented with little understanding of the dynamics of the soil food webs associated with these kinds of manipulations. Indeed, researchers often treat soil food web interactions as a "black box". They screen for epns that are most effective against their target insect under simplified laboratory conditions, and then apply them in the field in the hope that they will reduce pest numbers. When the results are positive, little effort is generally invested in the various potential short and long term ramifications of augmentation; and when the results are negative, the various potential causes of failure are rarely investigated. Nonetheless, some researchers have begun sorting out the dynamics of soil food webs involving epns in a few model systems, and a greater understanding of these food webs is slowly being achieved. This talk will briefly review our knowledge of such food webs and will focus especially on current research in Florida citrus groves.

Symposium. Thursday, 18:15. (209)

Modeling entomopathogenic nematode population patterns and processes in ecosystems

Casey Hoy, Parwinder Grewal, Robin Taylor, and J. J. Park

Entomopathogenic nematode populations are dynamic in space and time. Temporal and spatial patterns can be described with statistical models, and population processes can be described and explained mechanistically with analytical and simulation models. We will describe a set of models that have been used to explore the population dynamics of naturally occurring populations of entomopathogenic nematodes in an agricultural ecosystem. Statistical models have been used to examine the habitat characteristics associated with presence and abundance of nematodes, including associated arthropod host communities, and the spatial structure of nematode populations. Simulation models have been used to explore the population processes that could explain their patterns of abundance over time. Finally, analytical models have been used to explore metapopulation dynamics, which include specific patterns of abundance over time and space. Each of these approaches builds upon and connects the biological information that is generated in laboratory and field studies at the population, organismal and suborganismal levels. Taken together, they move us closer to an ability to manage entomopathogenic nematode populations in ecosystems.

CONTRIBUTED PAPERS, Thursday 16:15 - 18:15

BACTERIA 5

Contributed Paper. Thursday, 16:15. (210)

Adult non-biting midges: possible windborne carriers of Vibrio cholerae

Meir Broza

Faculty of Science and Science Education, University of Haifa, Oranim, Tiv'on, 36006, Israel

Vibrio cholerae is a waterborne bacterium native to the aquatic environment. There are over 200 known serogroups yet only two cause

cholera pandemics in humans. Direct contact of human sewage with drinking water, sea-born currents and marine transportation, represent modes of dissemination of the bacteria and thus the disease. The simultaneous cholera outbreaks that occur sometimes in distant localities within continental landmasses are puzzling. Here we present evidence that flying, non-biting midges (Diptera; Chironomidae), collected in the air, carry viable non-O1 V. cholerae. In laboratory experiments, flying adult midges that emerged from V. cholerae (O1) contaminated water transferred the green fluorescent protein (GFP)-tagged pathogenic bacteria from one laboratory flasks to another. Our findings show that aerial transfer by flying chironomids may play a role in the dissemination of V. cholerae in nature. A bio-climatic analysis showed a strong correlation between wind direction and intensity and the spato- temporal occurrence of cholera epidemics in the Indian sub continent and Africa, 1970-2006.

Contributed Paper. Thursday, 16:30. (211)

Isolation of an insect-active super toxin complex from a new species of the Yersinia

Mark R.H. Hurst1, Sandra M. Jones1, Trevor A. Jackson1 & Travis R. Glare1

1Biocontrol and Biosecurity, AgResearch, , Lincoln, New Zealand;

A unique species of the Enterobacteriaceae, a Yersinia sp., has been isolated from a diseased grass grub (Costelytra zealandica, Coleoptera: Scarabaeidae), field collected from New Zealand soils. The bacterium has broad host range killing a range of insect species notably members of the Coleoptera and Lepidoptera. After ingestion, the bacterium causes dissolution of the midgut epithelium followed by rapid invasion of the haemoceol where the bacterium multiplies rapidly leading to death of the insect within 72 hours post infection. Through transposon mutagenesis and DNA sequence analysis the main disease determinants of the bacterium have been identified and reside on a 32-kb Pathogenicity Island (PI). The region of the PI was deleted from the Enterobacteria's genome and the bacterium became avirulent. However virulence was restored by the introduction of a clone containing the PI. Sterile sonicated filtrates derived from only the wild type bacterium were able to cause virulence to larvae tested. The insect active genes represent a new member of the Toxin Complex family. To date, the purified toxin complex is able to cause mortality to many members of Coleoptera and Lepidoptera.

Contributed Paper. Thursday, 16:45. (212)

Exploring the use of RNAi for insect control

James A. Baum, William Clinton, Gregory R. Heck, Oliver Ilagan, Scott Johnson, Tichafa Munyikwa, Michael Pleau, Ty Vaughn, and James Roberts

Monsanto Company, 700 Chesterfield Parkway West, Chesterfield, Missouri 63017-1732

Since their first commercial introduction in 1996, insect-protected (Bt) crops have enjoyed widespread use in agriculture, particularly in the United States. The long-term success of the transgenic plant approach to insect protection depends on appropriate insect resistance management strategies as well the discovery and deployment of insecticidal agents with new modes of action. This presentation will describe our efforts to utilize the RNAi pathway to control infestations of insect pests on plants. We have demonstrated that the RNAi pathway can be triggered in a number of coleopteran species, including the western corn rootworm (WCR), by the mere ingestion of dsRNAs synthesized from target gene templates. DsRNAs from certain target genes can cause larval stunting and mortality in artificial diet feeding assays. Furthermore, corn plants expressing such dsRNAs exhibit protection from rootworm feeding damage.

Contributed Paper. Thursday, 17:00. (213)

Symbiotic bacteria, heat stress and pea aphid resistance to parasitoids

Jean-Frédéric Guay1, Dominique Michaud2, Conrad Cloutier1

1Département de biologie, 2Département de phytologie, Université Laval, Québec G1K 7P4

Abstract : Facultative symbiosis with bacteria greatly expands the ecological adaptation of aphids. Resistance to parasitoids is a clonal characteristic of the aphid Acyrthosiphon pisum implicating symbiotic bacteria of defensive value, specifically Hamiltonella defensa. We examine the hypothesis that thermosensibility of pea aphid resistance to the parasitoid Aphidius ervi is linked to the thermal resistance of their facultative symbionts. Experiments involve manipulating temperature for several A. pisum clonal lineages hosting different species of symbiotic bacteria, and characterizing their resistance level to A. ervi in terms of the functional integrity of their symbiotic associates.

Contributed Paper. Thursday, 17:15. (214)

Isolation, and Characterization of Two Toxin Complexes from Xenorhabdus nematophilus

Joel J. Sheets, Weiting W. Ni, Ignacio M. Larrinua and Tim D. Hey

Dow AgroSciences, Indianapolis, IN 46268

The toxin complex proteins from Xenorhabdus or Photorhabdus spp. bacteria represent novel insect active proteins that have potential to be used in transgenic crops to protect them from damaging insect pests. We have isolated two native toxin complexes from Xenorhabdus nematophilus. Complex 1 is composed of three different proteins; XptA2, XptB1xwi and XptC1xwi, having molecular weights of 280, 170, and 112 kDa, respectively. Complex 2 is composed of five different proteins, TcaC, TccB, TccA, TccC1 and an exochitinase. The molecular weight of XTC-2 was estimated to be between 1,150 – 1,300 kDa based upon migration on native PAGE and elution from size exclusion chromatography. Using size exclusion chromatography, electrophoretic mobility shift assays, and surface plasmon resonance spectroscopy, we show that XptA2, XptB1xwi and XptC1xwi bind together in a 4:1:1 stoichiometry. Recombinant XptB1xwi and XptC1xwi form a 1:1 binary complex that is very tightly associated. When added together in solution, these three proteins bind together post-translationally in an essentially irreversible process to form a toxin complex having the native 4:1:1 stoichiometry. The toxin complex has greater than 100-fold more insecticidal activity against susceptible insect pests than the recombinant XptA2 protein by itself.

Contributed Paper. Thursday, 17:30. (215)

Chemical mutagenesis of Heliothis virescens causes resistance to multiple Bacillus thuringiensis proteins

O. P. Perera, C. A. Blanco, R. E. Jackson, and C. A. Abel

Southern Insect Management Research Unit, USDA-ARS, Stoneville, Mississippi, USA

Newly emerged Heliothis virescens males from a Cry1Ac susceptible colony were subjected to chemical mutagenesis and mated with susceptible females from the same colony. Neonates of filial generations 1 and 2 were selected on artificial diet containing 1µg/ml Cry1Ac. A single larva survived the 7-day bioassay and was allowed to complete development. This resistant adult male was mated with susceptible females, and F1 progeny were inbred. Subsequent generations were selected on artificial diet containing 5 µg/ml Cry1Ac. LC50 values for Cry1Ac, Cry1F, and Cry2Ab were determined at F4 using dose mortality assays, and the mutated line demonstrated significantly higher levels of tolerance to these Bt proteins compared to the susceptible H. virescens line.

THURSDAY PM

Contributed Paper. Thursday, 17:45. (216)

Establishment of a proteomic approach to study brush border membrane proteins in Lepidopteran larvae

Yannick Pauchet and David G. Heckel.
Max Planck Institute for Chemical Ecology, Entomology department

Bacillus thuringiensis Cry toxins are widely used to control lepidopteran agricultural pest insects. Nevertheless, the evolution of resistance to transgenic plants producing those toxins poses a threat to their sustainable use in agriculture. The larval midgut brush border, as the primary target of Cry toxins, has been extensively studied in order to get clues on the molecular mechanisms triggering resistance to those toxins. There, toxin molecules interact with specific receptors, leading to the formation of pores in the plasma membrane, and finally to the lysis of midgut cells. Moreover, absence of specific receptors has been clearly shown to be responsible for resistance mechanisms. Because of the outstanding separating capabilities of 2D SDS-PAGE electrophoresis for complete proteomes, it would be advantageous to use it in comparative approaches to monitor changes between toxin-susceptible and -resistant larvae. Unfortunately, severe solubility problems hamper the analysis of many classes of proteins, especially membrane proteins which are the main component of the brush border membrane. The aim of this work was to establish a new approach based on 1D SDS-PAGE electrophoresis to resolve those membrane proteins and to evaluate the potential of this method in comparative studies of resistance mechanisms. Using this new approach, we will provide examples of BBMV proteome maps for several Lepidoptera and also an example of a comparison between Cry1Ac-susceptible and -resistant populations.

Contributed Paper. Thursday, 18:00. (217)

Bacillus thuringiensis: A very attractive bacterium for various biotechnological applications.

S. Jaoua, N. Zouari, . S. Tounsi, K. Jamoussi, H. Azzouz, S. Rouis, L. Abdelkefi Mesrati, R. Zribi, F. Kamoun, F. Driss, O. Kilani Feki, M. Dammak, M. Jemaa
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Bacillus thuringiensis, a gram positive soil bacterium, is characterized by the production of one or more insecticidal cytoplasmic protein crystal inclusions during sporulation.

The investigation of the 2 kind of insecticidal proteins of B. thuringiensis: the Cry delta-endotoxins and the Vip, vegetative insecticidal proteins was carried out. New Cry genes Cry1Aa, Cry1Ac and Cry2Aa have been evidenced. The investigation of their individual toxicity against the lepidopteran insect Ephestia kuehniella revealed that the best insecticidal formulation would be obtained by increasing Cry1Ac content in the crystals. Many strains showed strong insecticidal activities on lepidoptera and on diptera. Excellent LD50 of B. thuringiensis israelensis Tunisian strains against Culex pipiens of less than 5 ng/ml were obtained. But, we evidenced in a B. thuringiensis israelensis strain, for the first time, a DNA rearrangement in its 128kb pBtoxis plasmid and cry4A and cry10A deletions.

The investigation of B. thuringiensis VIP toxins revealed their coding gene was located on the same plasmid than that of cry1A and cry1Ac. Finally, the study of other B. thuringiensis biological activities of the laboratory strains collection, evidenced new bacteriocins, chitobiosidase and chitosanase, making this bacterium very attractive for various biotechnological applications.

CONTRIBUTED PAPERS, Thursday 16:15 - 19:30

MICROBIAL CONTROL 5

Contributed Paper. Thursday, 16:15. (218)

Comparative virulence of three hyphomycetous fungi against the bollworm, Helicoverpa armigera, employing topical versus per os inoculation techniques

Justin L. Hatting
ARC-Small Grain Institute, Private Bag X29, Bethlehem, 9700, South Africa

The increasing pest status in South Africa of the bollworm, Helicoverpa armigera, has prompted renewed interest in the use of bio-insecticides, especially due to suspected resistance developing against commonly used pyrethroids. Three hyphomycetous fungi, Beauveria bassiana (strain PPRI 8072), Nomuraea relyi (PPRI 7758) and Isaria fumosoroseus (3167) were compared in laboratory bioassays employing topical verus per os inoculation techniques. During initial assays, a per os dose of 5.625 x 105 conidia per larvae resulted in 100% and 95% mortality for N. relyi compared to 75% and 90% for I. fumosoroseus against 3rd and final instar larvae, respectively. Significantly lower mortality of only 20% was measured in both assays for the B. bassiana strain while control mortalities remained at 0%. In subsequent assays, the performance of these strains were again compared by including a topical application using the same conidial concentration against final instar (i.e., no ecdysis) larvae only. Results are discussed including the use of a novel per os inoculation substrate ensuring more accurate dose acquisition and subsequent quantification. Post-inoculation food intake as impacted upon by the various treatments is also discussed.

Contributed Paper. Thursday, 16:30. (219)

Integration of soil inoculation with Metarhizium anisopliae into bait-based technology for field suppression of Bactrocera invadens on mango

Sunday Ekesi & Nguya K. Maniania
International Centre of Insect Physiology and Ecology (icipe), PO Box 30772-00100 GPO, Nairobi, Kenya

One of the most damaging fruit flies attacking mango in Africa is the invasive fruit fly Bactrocera invadens. We screened several isolates of Beauveria bassiana and Metarhizium anisopliae for their pathogenicity to pupariating larvae and puparia in the laboratory and selected one of the most pathogenic isolates, M. anisopliae ICIPE 20, for soil application. Field trials were carried out for 2 seasons in smallholder mango orchard. Four treatments were applied: fungus combined with canopy application of commercial bait (GF-120), bait alone, fungus alone, and untreated control. During the first season, fruit infestation in the fungus plus bait treatment was 15% and yield was 9.8 tons while infestation was 24, 45 and 63% and yield was 6.4, 4.5 and 2.3 tons ha-1in the bait, fungus alone and control treatments, respectively. In the second season trial, 9% fruit infestation occurred in the bait plus fungus treatment and 21, 32 and 56% in the bait, fungus and control treatments, respectively. The respective mango yield was 10.5, 7.3, 5.1 and 3.3 in the bait plus fungus, bait alone, fungus alone and control treatments. Our result suggests that soil inoculation of M. anisopliae can be an important component of fruit fly IPM in mango agroecosystem.

Contributed Paper. Thursday, 16:45. (220)

Linkage and mapping analysis of a gene resistant to Bacillus thuringiensis Cry1Ab toxin in the silkworm Bombyx mori

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In Bombyx mori, resistance to Bacillus thuringiensis Cry1Ab toxin is controlled by the recessive resistance gene Bt-r (resistant to Bt Cry1Ab toxin), which is located on Chromosome 15 (Hara et al, 2003). The silkworm strains 606 and 401 are classified as being susceptible and highly resistant to Cry1Ab toxin, respectively. For the linkage and mapping analysis of Bt-r, 1,365 segregants of back-crosses (BC1) between 401-females and F1 (strain 401 x strain 606) males were used. A linkage map composed of 2,002 single nucleotide polymorphism (SNP) markers over the 28 linkage groups in the silkworm has previously been constructed (Yamamoto et al, 2005). This SNP linkage map is a powerful tool for map-based cloning of genes. BC1 larvae were exposed to Cry1Ab toxins at the first-instar stage. Genomic DNA was extracted from each of the surviving fifth-instar larvae and analyzed by segregation patterns of SNPs between strains 401 and 606 using the SNP markers on that map. Our results indicate that the resistant gene mapped to the 368.6-kbp region on Chromosome 15, since all surviving larvae showed a homozygous profile of strain 401 in their genotype within this region, and several surviving larvae showed heterozygous genotypes in other regions.

Contributed Paper. Thursday, 17:00. (221)

Integrated applications of Bacillus thuringiensis serovar. tenebrionis and Beauveria bassiana for biologically-based integrated pest management of Colorado potato beetle

Stephen P. Wraight and Mark E. Ramos
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Research conducted over the past decade has indicated a low level of synergism and a potentially high degree of complementarity between Bacillus thuringiensis (Bt)- and Beauveria bassiana (Bb)-based biopesticides applied for management of the Colorado potato beetle, Leptinotarsa decemlineata. In view of these findings, we have designed an integrated control program based on the hypothesis that early-season applications of the fast-acting (toxic) Bt will provide rapid control of early-instar larval populations (protecting the crop from defoliation), while subsequent applications of the slow-acting Bb against large larvae that survive Bt intoxication will provide control of mature larvae after they enter the soil to pupate (reducing the summer adult and over-wintering beetle populations). Demonstration trials of this integrated biocontrol program were initiated in 2006 in 0.17 ha plots of potatoes at the Cornell Entomology Research Farm in Freeville, NY. Novodor FC® (Bt tenebrionis) and BotaniGard ES® (Bb strain GHA) were applied at rates of 5 L (103 million Leptinotarsa units) and 1.25 L (2.5 E 13 conidia) in 468 L water per ha, respectively. A program of three spray applications: 1) Bt alone applied at time of ca. 50% hatch, 2) Bt + Bb (tank-mix) applied vs. second-instar larvae, and 3) Bb alone applied vs. late-instar larvae was compared to a biorational chemical control check (spinosad formulated as Entrust®) and an untreated control. Levels of defoliation, potato yields, and reductions in first-generation (summer) adult beetles were statistically equivalent to those achieved with spinosad.

Contributed Paper. Thursday, 17:15. (223)

Efficacy trials of Beauveria bassiana and Metarhizium anisopliae for Pieris rapae (Lepidoptera: Pieridae) control on commercial cabbage cultures

Garcia G. Cipriano, Gonzalez M. Berenice and Chairez H. Isaias.
CIIDIR-COFAA-IPN Unidad Durango. Durango, Dgo. México.

Every year the cabbage worm (CW) Pieris rapae (L), causing important damages in cabbage cultures in Durango, Mexico; for this reason were carried out the field trials at concentrations of 1.2x1012, 1.2x109 and 1.2x106 conidia/ha of Beauveria bassiana BbPM native strain, a commercial formulations of B. bassiana (Bea-SinTM) and Metarhizium anisopliae (Meta-SinTM) sprayed onto cabbage plants during 2005-2006. Spores aerial of B. bassiana BbPM were produced using a diphasic fermentation procedure and formulated with CeliteTM. A commercial cabbage plots were used to apply the bio-insecticides, as well as a chemical insecticide. Larvae died of CW were sampling at 7 days intervals. Statistical significant differences were found between treatments and concentrations ($F = 5458$, $p \leq 0.001$), but there were not significant statistical differences were found on sampling larvae on days 7 and 14, only on days 21 and 28 ($LSD = 1.972$). The plots treated with B. bassiana (BbPM) and Bea-SinTM at dose of 1.2x1012 conidia/ha provided a significant population decrease, with a mean larvae mortality of 93.3% and 91% respectively, 7 days after spraying, also M. anisopliae strain Meta-SinTM had decreased larvae concentrations (64%) on day 14. Our study confirmed the potential of field application of these products for control of CW larvae.

Contributed Paper. Thursday, 17:30. (224)

Biology of Sunn Pest (Eurygaster integriceps) (Hemiptera: Scutelleridae) relevant to control with a mycoinsecticide

Dave Moore1, Steve Edgington1,
Mustapha El Bouhssini2, Ziad Sayyadi2,
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2 International Centre for Agricultural Research in the Dry Areas (ICARDA), P.O. Box 5466, Aleppo, Syria.

A series of experiments with oil formulations of Beauveria bassiana to control Eurygaster integriceps demonstrated great potential for control, but this potential was not realised in the field. Field application was good, showing distribution through the wheat crop and 70% of Sunn Pest received direct hits. Behavioural studies demonstrated far greater activity of Sunn pest than previously suspected, suggesting that Sunn Pest would also be very likely to take up spores from sprayed vegetation. However, mortality assessments, conducted solely in the field, showed no effect upon Sunn Pest numbers. Other than treated Sunn Pest being heavier than untreated, there were no observable effects of the treatments. The likely reasons for this and implications for further work will be discussed.

Contributed Paper. Thursday, 17:45. (225)

Beauveria bassiana plus chemical attractant: A new approach against pine sawyer?

Zengzhi Li, Meizhen Fan Sibao Wang Degui Ding
Department of Forestry, Anhui Agricultural University, Hefei, Anhui230036, P.R.China

The pine sawyer, Monochamus alternatus, is the main vector of pine wilt disease caused by Bursaphelenchus xylophilus. It was discovered in China in 1982 and has caused tremendous economic and ecological loss in most provinces throughout Southern China. In Eastern China, the sawyer occurs one generation each year. The adults emerge not synchronously, from May through August, making spray of both chemicals and conidia of Beauveria bassiana, a common pathogen of the sawyers poorly effective in pine stands. Non woven fabric bands impregnated with B. bassiana were used in plots, resulting in effective suppression of sawyer populations. The method of binding each tree trunk with the fungal bands, however, is costly, impractical in mountainous area and unacceptable.

Chemical attractants were used one tree every hectare, 10 trees around each attractant were bound with the fungal band at 2 M from ground, one for each tree. Some adults were introduced from pine stands not treated with B. bassiana into cages in treatment plots while some adults attracted in the treatment plots were taken away,

reared for checking infection in laboratory. The adults introduced 2 day after treatments were infected up to 62% and those introduced 20 days after treatment up to 32%. The adults reared in the laboratory were infected up to 60% 2 days after treatments and up to 23% 20 days after treatments. Meanwhile, lifespan of the adults in treatments were shortened by 1/3 to 1/2.

The oviposition scars were increased extremely significantly, suggesting that the trapping was very obvious. However, the rates of invading holes to oviposition scars and larva number to invading holes of the larvae of the treatments were both significantly lower than those of the non treatment control, and infection rates of the treatments were significantly higher than that of the control.

After one year, the mortality of pine trees declined by over 60% as compared to that in the control area.

In the meantime, an inventory investigation of local entomopathogenic fungi was made before treatment and cadavers and soil samples were collected and isolated after treatment. All the 599 isolates recovered in the plots before the treatment and within one year after the treatment were tested for their 28S rDNA Group I intron. Twenty-four types of the intron were identified and 462 isolates were attributed to Type BBBA, the type of the released isolate, which did not appear in the inventory of local B. bassiana isolates before the treatment.

All the evidences suggest that the sawyers were successfully attracted to around the attractants, infected substantially by B. bassiana artificially, and epicenters due to the use of the fungal bands did form and the sawyer population was suppressed substantially, indicating that the combined use of the fungal bands and chemical attractants is an effective approach for the sawyer control.

AUTHOR INDEX

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